BIOMARKERS FOR THE EFFICACY OF CALCITONIN AND PARATHYROID HORMONE TREATMENT

FIELD OF THE INVENTION

[0001] This invention relates generally to the analytical testing of tissue samples *in vitro*, and more particularly to aspects of gene expression profiling concerning calcium regulation.

BACKGROUND OF THE INVENTION

[0002] Calcium is essential for many cellular processes in the body and especially important for bone metabolism. The level of calcium in the body is carefully maintained by an endocrine control system. Two of the hormones in this endocrine control system are calcitonin and parathyroid hormone.

[0003] Calcitonins, which are polypeptide hormones of about 32 amino acids, are endogenous regulator of calcium homeostasis and can be used as anti resorptive agents for the treatment of hypocalcaemia-associated disorders. Calcitonin is produced in the parafollicular cells (C cells) of the thyroid gland. Various calcitonins, including *e.g.* salmon and eel calcitonin, are commercially available and are commonly employed in the treatment of *e.g.* Paget's disease of bone, malignant hypocalcaemia and post-menopausal osteoporosis. Pondel M, *Intl. J. Exp. Pathol.* 81(6): 405-22 (2000). A version of calcitonin (Miacalcin®) is available as a nasal spray.

[0004] Parathyroid hormone (PTH) is a polypeptide of 84 amino acids. Parathyroid hormone regulates bone remodelling and Ca^{2+} homeostasis. Parathyroid hormone is also a known paracrine activator of osteoclast differentiation and activity. PTS893 [SDZ PTS 893; Leu8, Asp10, Lys11, Ala16, Gln18, Thr33, Ala34 human PTH 1-34 [hPTH(1-34)]] is a 34 amino acid parathyroid analogue that enhances bone mass and biomechanical properties. Kneissel M *et al., Bone* 28: 237-50 (March 2001); Stewart AF *et al., J. Bone. Miner. Res.* 15(8): 1517-25 (August 2000); Thomsen JS *et al., Bone* 25(5):561-9 (November 1999).

[0005] Calcitonin and parathyroid hormone are known to interact in a complex and interdependent manner, but the understanding of how calcitonin and parathyroid hormone interact has been incomplete. Calcitonin inhibitory effects on osteoclast resorptive activity, and renal tubular calcium resorption have been well documented. However, potential

calcitonin effects on osteoblasts and interactions with any other skeletal-metabolism-related factors have remained controversial.

[0006] Multi-organ gene profiling analysis would provide a better picture of the changes induced by a compound on the whole organism and also give a new perspective to the understanding of the pharmacology of hormones. Genomics technologies are a source of the new hypothesis-generating capabilities that are now empowering biomedical researchers. In the context of drug development, they provide with a new perspective to the understanding of the pharmacology of drugs. Accordingly, there is a need in the art for an organism-wide understanding of the activity of calcitonin and parathyroid hormone.

SUMMARY OF THE INVENTION

[0007] The invention provides a response to the need in the art. Multi-organ gene profiling analysis provides with a complete picture of the changes induced by a compound on the whole organism, and gives a new perspective to the understanding of the pharmacology of drugs. In one aspect, the invention provides the first description of the molecular mechanisms of action of hormonal-mediated bone remodelling by salmon calcitonin by gene profiling analysis. The known mechanisms of action of calcitonin as anti-resorptive agent could be reconstructed at the molecular level. Effects on effectors and pathways linked to bone remodelling activities – BMPs, IGFs, extracellular matrix components and VEGF - were also observed. These results support the role of calcitonin as an anabolic agent. In another aspect, the invention provides the first reconstruction of the molecular mechanisms of action of a pharmacological agent on one of its target tissues in an intact primate animal model, by evaluating the gene expression changes induced by salmon calcitonin or the parathyroid hormone analogue PTS893 on bone in cynomolgus monkeys, to elucidate the molecular mechanisms of action mediating their effects. Gene profiling analysis allowed the reconstruction of the pathways involved in calcitonin signal transduction, triggered by protein-G-linked-receptor stimulation and their influence on cell cycle, as indicated by the changes observed in cyclins. *In vivo* gene-profiling expression studies allow the identification of the molecular mechanisms underlying a pharmacological effect.

[0008] In one embodiment, the invention provides for the use of calcitonin in the manufacture of a medicament for the treatment of a condition for which treatment with an anabolic agent is indicated. In one embodiment, the condition is atherosclerosis.

[0009] The invention also provides for the use of calcitonin in the manufacture of a medicament for the treatment of disorders of calcium metabolism in a selected patient population, where the patient population is selected on the basis of the gene expression profile indicative of calcitonin efficacy by the patient to whom calcitonin is administered. In one embodiment, the calcitonin is salmon calcitonin. The invention further provides for the use of a parathyroid hormone or parathyroid hormone analogue in the manufacture of a medicament for the treatment of disorders of calcium metabolism in a selected patient population, where the patient population is selected on the basis of the gene expression profile indicative of parathyroid hormone or parathyroid hormone analogue efficacy by the patient to whom parathyroid hormone or parathyroid hormone analogue is administered. In one embodiment, the hormone analogue is PTS893. In one embodiment, the medicament is administered in a therapeutic dose prior to determining the gene expression profile by the patient. In another embodiment, the medicament is administered in a sub-therapeutic dose prior to determining the gene expression profile by the patient.

[0010] The invention also provides a method for treating a condition in a subject, wherein the condition is one for which administration of a calcitonin, parathyroid hormone, a parathyroid hormone analogue or a combination thereof is indicated. The method involves, first administering a compound of interest to the subject (*e.g.*, a primate subject) and then obtaining the gene expression profile of the subject following administration of the compound. The gene expression profile of the subject is compared to a biomarker gene expression profile. The biomarker gene expression profile is indicative of efficacy of treatment by a calcitonin, parathyroid hormone, a parathyroid hormone analogue or a combination thereof. In one embodiment, the biomarker gene expression profile is the baseline gene expression profile of the subject before administration of the compound. In another embodiment, the biomarker gene expression profile is the gene expression profile or average of gene expression profiles of a vertebrate to whom calcitonin (*e.g.*, salmon calcitonin) or parathyroid hormone or a parathyroid hormone analogue (*e.g.*, PTS893) has been administered. A similarity in the gene expression profile of the subject to whom the

compound was administered to the biomarker gene expression profile is indicative of efficacy of treatment with the compound.

[0011] Accordingly, the invention provides biomarkers for the efficacy of treatment of a condition for which calcitonin, parathyroid hormone or a combination thereof is indicated. Among the biomarkers are the expression profiles of the genes for Y-box binding protein, bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), vascular endothelial growth factor (VEGF), α-2-HS glycoprotein (AHSG), osteoclast stimulating factor (OSF), nuclear receptors (steroid/thyroid family) and others.

[0012] The invention provides methods for determining a subject for inclusion in a clinical trial, based upon an analysis of biomarkers expressed in the subject to be treated. The compound to be tested is administered to the subject. In one embodiment, the compound to be tested is administered in a sub-therapeutic dose. Then, the gene expression profile of the subject following administration of the compound is obtained. The subject may be included in the clinical trial when the gene expression profile of the subject to whom the compound was administered is similar to a biomarker gene expression profile indicative of efficacy of treatment by a calcitonin, parathyroid hormone, a parathyroid hormone analogue or a combination thereof. The subject may be excluded from the clinical trial when the gene expression profile of the subject is dissimilar to the biomarker gene expression profile indicative of efficacy of treatment. Such similarities or dissimilarities are observable to those of skill in the art.

[0013] The invention also provides clinical assays, kits and reagents for determining treatment efficacy of a condition for which administration of a calcitonin, parathyroid hormone or a parathyroid hormone analogue is indicated. In one embodiment, the kits contain reagents for determining the gene expression of biomarker genes, by hybridization. In another embodiment, the kits contain reagents for determining the gene expression of biomarker genes, by the polymerase chain reaction.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0014] This invention is based upon an understanding of the effects of administration to a subject of calcitonin (*e.g.*, salmon calcitonin; SEQ ID NO:1) or parathyroid hormone (SEQ ID NO:2) or an analogue thereof (*e.g.*, PTS893; SEQ ID NO:3). A multi-organ gene profiling

analysis of the results of an administration to a subject of salmon calcitonin or a parathyroid hormone analogue provides biomarkers of calcitonin treatment efficacy and parathyroid hormone or parathyroid hormone analogue treatment efficacy. As used herein, a subject is a vertebrate. In one embodiment, the vertebrate is a mammal. In a more particular embodiment, the subject is a primate, *e.g.,* a cynomolgus money or a human.

[0015] The analysis provided here globally describes the molecular mechanisms of action of salmon calcitonin and the PTS893 in changing the ribonucleic acid (RNA) content in different organs by multi-organ gene profiling analysis in primates. The RNA content of the cell, the "transcriptome" is a reflection of the cell functions and status. Inside an individual cell or an organ, the expressions of the different elements of a transcriptome are not independent. The change in expression level can trigger a series of events that will lead finally to another modification of the transcriptome. These interdependent events are described in terms of pathways. Because the changes in the different functions inside a cell are tightly interconnected, the changes in different organs inside the organism are linked. Applying gene profiling to different organs submitted to the same treatment gives an improved overview of the effects and the modifications of the physiological status. As shown herein, this is particularly the situation when multi-organ profiling analysis of pleiotropic compounds, such as calcitonin, is to be performed. Indeed, the global signature described for calcitonin is reflected not only in the main target organ (*i.e.,* bone) but also in the other organs analyzed herein.

[0016] In this multi-organ gene profiling analysis, the known mechanisms of action of calcitonin as an anti-resorptive agent and the parathyroid hormone PTS893 as a paracrine activator of osteoclast differentiation and activity could be reconstructed at the molecular level. The calcitonin inhibitory effect on osteoclasts could be reconstructed, with changes affecting, among others, genes for PU.1 (SPI1; SpiB; SEQ ID NO:4), colony stimulating factor (CSF-1 (SEQ ID NO:6); differentiation and survival) carbonic anhydrase (SEQ ID NO:8), H^+-ATPases, cathepsin K (resorptive activity) tubulins, PAK4 (motility). Effects on effectors and pathways linked to bone remodelling activities (bone morphogenetic proteins (BMPs), fibroblast growth factors (FGFs), insulin-like growth factors (IGFs), extracellular matrix components, steroid hormones, vascular endothelial growth factor (VEGF) and α-2-HS glycoprotein (AHSG)) were also observed, shared in many cases by both salmon calcitonin

and PTS893. Interestingly, salmon calcitonin also regulates the expression of the gene codifying for osteoclast stimulating factor (OSF), and cystatin. Also interestingly, PTS893 also regulates the genes implicated in osteoclast differentiation and survival (SPI1, CSF-1, monocyte to macrophage differentiation-associated protein (MMD)). PTS893 also produced a strong up-regulation on nuclear receptors (steroid/thyroid family). Accordingly, these results support the role of calcitonin as anabolic agent.

[0017] Calcitonin is presently used in the treatment of systemic skeletal diseases characterized by high bone mass which are a consequence of imbalance between bone formation (anabolic) and resorption of bone, with the former predominating. Calcitonin promotes the synthesis of bone morphogenetic protein-2 (BMP-2), which is known to be a potent anabolic agent. The evidence is strong that when calcitonin gets to bone cells, they can have an anabolic effect by increasing production of BMP-2. Thus calcitonin can be used in a method of treating an individual to adjust a subject's bone mineral density.

[0018] This the first approach to characterise in an *in vivo* model the effects of calcitonin on bone metabolism by gene expression profiling. The calcitonin inhibitory effect on osteoclasts could be reconstructed, with changes affecting genes as carbonic anhydrase, H^+-ATPases and cathepsin K. Salmon calcitonin also seemed to regulate the expression of the gene codifying for cystatin, being this effect described here for the first time. Salmon calcitonin has also modulating effects on genes affecting the direct, autocrine, paracrine and endocrine regulation of the mesenchymal cell functions such as pleiothropin, periostin, fibroblast growth factor, transforming growth factor betas (TGF-betas), insulin-like growth factors/binding proteins (IGFs/IGFBPs), bone morphogenetic proteins (BMPs), Vascular Endothelial Growth Factor (VEGF), Tumour Necrosis Factor (TNF), neurochondrin, follistatin-like 3, or parathyroid hormone receptor. It also regulates the synthesis and degradation of extracellular matrix components (collagens, osteopontin, osteocalcin, dermatopontin, chondroadherin, glypican or syndecan) and enzymes. Salmon calcitonin also influenced some aspects of bone mineralization, since changes in dentin were observed.

[0019] As provided herein, calcitonin can also be used as an anabolic agent in the treatment of other conditions where anabolism or tissue growth is therapeutically desirable. Such a condition is atherosclerosis, an atheromatous disease in which the atheromatous plaque is complicated by fibrosis and calcification.

[0020] Moreover, the invention provides biomarkers of the efficacy of calcitonin or parathyroid hormone treatment. As used herein, a gene expression profile is diagnostic for determining the efficacy of treatment when the increased or decreased gene expression is an increase or decrease (*e.g.*, at least a 1.5-fold difference) over the baseline gene expression following administration of the compound (*i.e.*, the biomarker gene expression profile is the baseline gene expression profile of the subject before administration of the compound). Alternatively or in addition, the gene expression profile is diagnostic for determining the efficacy of treatment as compared with treatment of calcitonin (*e.g.*, salmon calcitonin) or parathyroid hormone or parathyroid hormone analogues (*e.g.*, PTS893) when the gene expression profile of the treated subject is comparable to a standard biomarker gene expression profile. In one embodiment, the standard biomarker gene expression profile is the gene expression profile or average of gene expression profiles of a vertebrate to whom a calcitonin, parathyroid hormone, a parathyroid hormone analogue of a combination thereof has been administered, this profile or profile being the standard to which the results from the subject following administration is compared. Such an approach, which contains aspects of therapeutics and diagnostics, is termed "theranostic" by many of those of skill in the art.

[0021] In one embodiment, the subject is a vertebrate. In a particular embodiment, the vertebrate is a mammal. In a more particular embodiment, the mammal is a primate, such as a cynomolgus monkey or a human. As used herein, the administration of an agent or drug to a subject or patient includes self-administration and the administration by another.

[0022] As used herein, a gene expression profile is diagnostic of the efficacy of calcitonin or parathyroid hormone treatment when the increased or decreased gene expression is an increase or decrease (*e.g.*, at least a 1.5-fold difference) over the baseline gene expression following administration of a calcitonin or of parathyroid hormone or an analogue. As used herein, a gene expression pattern is "higher than normal" when the gene expression (*e.g.*, in a sample from a treated subject) shows a 1.5-fold difference (*i.e.*, higher) in the level of expression compared to the baseline samples. A gene expression pattern is "lower than normal" when the gene expression (*e.g.*, in a sample from a treated subject) shows a 1.5-fold difference (*i.e.*, lower) in the level of expression compared to the baseline samples.

[0023] Techniques for the detection of gene expression of the genes described by this invention include, but are not limited to northern blots, RT-PCT, real time PCR, primer

extension, RNase protection, RNA expression profiling and related techniques. Techniques for the detection of gene expression by detection of the protein products encoded by the genes described by this invention include, but are not limited to, antibodies recognizing the protein products, western blots, immunofluorescence, immunoprecipitation, ELISAs and related techniques. These techniques are well known to those of skill in the art. Sambrook J *et al.*, *Molecular Cloning: A Laboratory Manual, Third Edition* (Cold Spring Harbor Press, Cold Spring Harbor, 2000). In one embodiment, the technique for detecting gene expression includes the use of a gene chip. The construction and use of gene chips are well known in the art. See, U.S. Pat Nos. 5,202,231; 5,445,934; 5,525,464; 5,695,940; 5,744,305; 5,795,716 and 5,800,992. See also, Johnston, M. *Curr Biol* 8:R171-174 (1998); Iyer VR *et al., Science* 283:83-87 (1999) and Elias P, "New human genome 'chip' is a revolution in the offing" *Los Angeles Daily News* (October 3, 2003).

[0024] The gene expression profile may include one or more genes selected from the group of acid phosphatase 1 isoform a; activin A receptor type II like 1; activin A type IIB receptor precursor; activin beta C chain; alpha 2 HS glycoprotein; amelogenin; annexin V; arylsulfatase E precursor; ATPase H(+) vacuolar; ATPase H(+) vacuolar subunit; ATPase, H+ transport, lysosomal; ATPase, H+ transporting, lysosomal; ATPase, H+ transporting, lysosomal; biglycan; bone morphogenetic protein 1; bone morphogenetic protein 10; bone morphogenetic protein 2A; bone morphogenetic protein 5; bone morphogenetic protein 6 precursor; calcium binding protein 1 (calbrain); calcium/calmodulin dependent protein kinase (CaM kinase) II gamma; calreticulin; cAMP responsive element modulator (CREM); carbonic anhydrase I; carbonic anhydrase II; cartilage oligomeric matrix protein precursor; cathepsin K; cathepsin W; CDC like kinase 1; CDC like kinase 2 isoform hclk2/139; chondroitin sulphate proteoglycan 2 (versican); chondroitin sulphate proteoglycan 3 (neurocan); chorionic somatomammotropin hormone 1; chymotrypsin C (caldecrin); collagen type 1 and PDGFB fusion transcript; collagen type II alpha 1; collagen type III alpha 1; collagen type IV alpha 2; collagen type IX alpha1; collagen type VI alpha 1; collagen type VI alpha 2 (AA 570 998); collagen type XI alpha 1; collagen type XI alpha2; collagen type XI alpha2; collagen, type I, alpha 2; collagen, type IV, alpha 1; collagen, type IX, alpha 2; collagen, type V, alpha 2; collagen, type VI, alpha 1; collagen, type VI, alpha 1 precursor; collagen, type XVI, alpha 1; collagen, type XVI, alpha 1; collagenase 3 (matrix metalloproteinase 13); connective tissue

growth factor; cyclin A2; cyclin B1; cyclin D2; cyclin E2; cyclin dependent kinase 5; cyclin dependent kinase 5, regulatory subunit 1 (p35); cyclin dependent kinase 6; cyclin dependent kinase inhibitor 1A (p21, Cip1); cystatin B (stefin B); cytokine inducible kinase; death associated protein kinase 1; death associated protein kinase 3; dentin matrix acidic phosphoprotein 1 (DMP1); dual specificity phosphatase 9; dystrophia myotonica protein kinase; ectonucleotide pyrophosphatase/ phosphodiesterase 1; ectonucleotide pyrophosphatase/ phosphodiesterase 1; endothelial differentiation, G protein coupled receptor 6 precursor; oestrogen receptor; oestrogen receptor; oestrogen receptor related protein; oestrogen responsive B box protein (EBBP); fibroblast activation protein; fibroblast growth factor 1 (acidic); fibroblast growth factor 18; fibroblast growth factor 4; fibroblast growth factor receptor; follistatin like 1; follistatin like 1; glutamate receptor, metabotropic 1; GPI1 N acetylglucosaminyl transferase component Gpi1; granulocyte macrophage colony stimulating factor (CSF1); growth arrest and DNA damage inducible, alpha; growth factor receptor bound protein 10; heparan sulphate proteoglycan 2 (perlecan); inositol 1,4,5 triphosphate receptor, type 1; inositol 1,4,5 triphosphate receptor, type 1; inositol 1,4,5 triphosphate receptor, type 2; inositol 1,4,5 trisphosphate 3 kinase isoenzyme; inositol polyphosphate 4 phosphatase type I beta; inositol polyphosphate 5 phosphatase; inositol(myo) 1(or 4) monophosphatase 1; inositol(myo) 1(or 4) monophosphatase 2; insulin like growth factor (IGF II); insulin like growth factor 2 (somatomedin A); insulin like growth factor binding protein; insulin like growth factor binding protein 2; insulin like growth factor binding protein 3; insulin like growth factor binding protein 5; insulin like growth factor binding protein 2; insulin like growth factor II precursor; insulin like growth factor II precursor; integrin alpha 10 subunit; interleukin 1 receptor associated kinase; Janus kinase 3; LIM protein (similar to rat protein kinase C binding enigma); lysyl oxidase like protein; MAD, mothers against decapentaplegic homolog 3; MAGUKs (membrane associated guanylate kinase homologues; MAP kinase kinase kinase (MTK1); MAPK13: mitogen activated protein kinase 13; MAPK8IP1: mitogen activated protein kinase 8 interacting protein 1; MEK kinase; metalloproteinase; mitogen activated protein kinase 1; mitogen activated protein kinase 8; mitogen activated protein kinase kinase 1; mitogen activated protein kinase kinase kinase kinase 4; mitogen activated protein kinase activated protein kinase 2; mitogen activated protein kinase activated protein kinase 3; MMD: monocyte to macrophage differentiation associated; neurochondrin; nuclear

factor of activated T cells, cytoplasmic, calcineurin dependent 1; OS 4 protein (OS 4); OSF 2os osteoblast specific factor 2 (periostin); osteoclast stimulating factor (OSF); PAK4; PDGF associated protein; phosphatidylinositol 4 kinase, catalytic, beta polypeptide; phosphatidylinositol glycan, class L; phosphatidylinositol polyphosphate 5 phosphatase, isoform b; phosphatidylinositol 4 phosphate 5 kinase isoform C (1); phosphatidylinositol 4 phosphate 5 kinase, type I, beta; phosphatidylinositol 4 phosphate 5 kinase, type II, beta; phosphatidylinositol glycan class C (PIG C); phosphodiesterase 4A, cAMP specific; phosphodiesterase 4D, cAMP specific (dunce (Drosophila) homolog phosphodiesterase E3); phosphodiesterase IB, calmodulin dependent; phosphoinositide 3 kinase; phosphoinositide 3 kinase, catalytic, gamma polypeptide; phosphoinositide 3 kinase, class 3; phospholipase C b3; phospholipase C, beta 4; phospholipase D; phosphotidylinositol transfer protein; PKD2 Protein kinase D2; preprocollagen type I alpha 2; preprocollagen type I alpha1; procollagen alpha 1 type II; procollagen lysine 5 dioxygenase; procollagen proline, 2 oxoglutarate 4 dioxygenase (proline 4 hydroxylase), alpha polypeptide I; progestagen associated endometrial protein (placental protein 14, pregnancy associated endometrial alpha 2 globulin, alpha uterine protein); prolidase (imidodipeptidase) PEPD; proliferating cell nuclear antigen; prolyl 4 hydroxylase beta; protease, serine, 11 (IGF binding); proteasome (prosome, macropain) subunit, beta type, 10; protein inhibitor of activated STAT X; protein kinase 1 PCTAIRE; protein kinase C substrate 80K H; protein kinase C, alpha; protein kinase, cAMP dependent, catalytic, gamma; protein kinase, cAMP dependent, regulatory, type I, beta; protein kinase, cAMP dependent, regulatory, type II, alpha; purinergic receptor P2Y, G protein coupled, 11; RAC2 Ras related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2); receptor tyrosine kinase DDR; retinoid X receptor gamma; ribosomal protein S6 kinase; ribosomal protein S6 kinase, 90kD, polypeptide 3; SCAMP1: secretory carrier membrane protein 1 (vesicular transport); secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T lymphocyte activation 1); serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 2; serine/threonine kinase 38; serine/threonine protein kinase; SF 1; Steroidogenic factor 1; signal transducer and activator of transcription 1; signal transducer and activator of transcription 2, 113kD; signal transducer and activator of transcription 5A; signal transducer and activator of transcription 5A; signal transducer and activator of transcription 6 (STAT6); Smad 3; Smad anchor for receptor activation, isoform 1;

Smad5; SMAD6 (inhibits BMP/Smad1 (MADH1); SNF 1 related kinase; SpiB transcription factor (SPI1/PU.1 related); Stat5b (stat5b); Ste20 related serine/threonine kinase; TEIG; TGFB inducible early growth response; TGFB inducible early growth response; TIEG; TGFB1 induced anti apoptotic factor 1; TGF beta induced apoptosis protein 12; TGF beta precursor; TGF beta superfamily protein; Tob; tousled like kinase 1; transforming growth factor, beta receptor III (betaglycan, 300kD); transforming growth factor beta 3 (TGF beta 3); TRIO: triple functional domain (PTPRF interacting); tubulin alpha 1; tubulin alpha 3; tubulin alpha isotype H2 alpha; tubulin beta 2; tubulin beta 3; tubulin beta 4; tubulin beta, cofactor D; type VI collagen alpha 2 chain precursor; ubiquitin carrier protein E2 C; vascular endothelial growth factor; vascular endothelial growth factor; vascular endothelial growth factor B; and Y box binding protein 1.

[0025] As used herein, the administration of an agent or drug to a subject or patient includes self-administration and the administration by another.

[0026] *Calcitonin.* The term "calcitonin" includes not only the naturally occurring calcitonins, but also their pharmaceutically active derivatives and analogues, *e.g.* in which one or more of the peptide residues present in the naturally occurring product is replaced, or in which the N- or C-terminal is modified. Preferred calcitonins for use in accordance with the invention are salmon, human and porcine calcitonins and Elcatonin. All of these compounds are commercially available and have been extensively described, together with their pharmaceutical properties, in the literature. *See*, U.S. Pat. Nos. 5,733,569 and 5,759,565, the contents of which are incorporated by reference.

[0027] The amount of calcitonin to be administered in accordance with the method of the invention and hence the amount of active ingredient in the composition of the invention depends on the particular calcitonin chosen, the condition to be treated, the desired frequency of administration and the effect desired.

[0028] The bioavailability for calcitonins, in particular salmon calcitonin, as determined in terms of blood plasma concentration following nasal administration is high, generally of the order of *ca.* 50% of levels achieved on intra-muscular injection. Accordingly administration in accordance with the invention will appropriately be effected so as to give a dosage rate of the order of two times or more, *e.g.* from about two to four times the dosage rate required for treatment via intra-parietal, *e.g.* intra-muscular, administration. Information regarding the

administration of Miacalcin® (calcitonin-salmon) nasal spray is available in the Miacalcin® Prescribing Information (Novartis, November 2002).

[0029] For intra-muscular injection, individual dosages of *ca.* 50 to 100 MRC units are applied at a rate of from *ca.* one time daily to *ca.* three times weekly. For nasal administration in accordance with the present invention, treatment will therefore suitably comprise administration of dosages of from about 50 to about 400 MRC units, more preferably from about 100 to about 200 MRC units at a frequency of from about one time daily to about three times weekly. Conveniently dosages as aforesaid will be administered in a single application, *i.e.* treatment will comprise administration of single nasal dosages comprising about 50 to about 400 MRC units, preferably about 100 to about 200 MRC units, calcitonin. Alternatively such dosages may be split over a series of *e.*g. two to four applications taken at intervals during the day, the dosage at each application then comprising about 10 to about 200 MRC units, preferably about 25 to about 100 MRC units.

[0030] The total composition quantity administered at each nasal application suitably comprises from about 0.05 to 0.15 ml, typically about 0.1 ml, *e.g.* 0.09 ml. Compositions for use accordingly suitably comprise from about 150 to about 8,000, preferably from about 500 to about 4,000, more preferably from about 500 to about 2,500, and most preferably from about 1,000 to about 2,000 MRC units calcitonin, *e.g.* salmon calcitonin, per ml.

[0031] The term "calcitonin" also encompasses active peptide analogues and mimetics, such as described for example, in U.S. Pat. Nos. 5,719,122, 5,175,146, and 5,698,6721. See, U.S. Pat. Appln. 2003015815. The "calcitonin superfamily" consists of calcitonin, calcitonin gene-related peptide (CGRP), and amylin. Calcitonin and CGRP derive from the CT/CGRP gene, in humans. Alternative splicing of the primary RNA transcript leads to the translation of CGRP and CT peptides in a tissue-specific manner. CGRP (a 37-amino-acid neuropeptide) and its receptors are widely distributed in the body. Amylin (a 37-amino-acid peptide) is generated from a gene located on chromosome 12 (thought to be an evolutionary duplication of chromosome 11) and shares 46% amino acid sequence homology with CGRP and 20% with human calcitonin. The term "calcitonin gene-related peptide" or "CGRP" includes native CGRP, preferably human CGRP, and its active analogues. CGRP is known to have a variety of roles in bone formation. The term "amylin" includes native amylin, typically from a human source, and its pharmaceutically active analogues. The hormone is known to induce bone-

mass formation through a variety of mechanisms. "Calcitonin-like agents" include "calcitonin," "CGRP," and "amylin." See, U.S. Pat. Appln. 003015815.

[0032] *Parathyroid hormone*. The term "parathyroid hormone" refers to parathyroid hormone, fragments or metabolites thereof and structural analogues thereof which can stimulate bone formation and increase bone mass. Also included are parathyroid hormone related peptides and active fragments and analogues of parathyroid related peptides. See, U.S. Pat. Nos. 4,086,196, 5,001,223, 6,541,450 and 6,649,657 and published PCT patent applications WO 94/01460 and WO 93/06845. Parathyroid hormone functional activity is readily determined by those skilled in the art according to standard assays. A variety of these compounds are described and referenced below, however, other parathyroid hormones will be known to those skilled in the art. Exemplary parathyroid hormones are disclosed in the references cited in U.S. Pat. Nos. 6,541,450 and 6,649,657, the entire contents of which are incorporated by reference. The utility of parathyroid hormones as medical agents in the treatment of conditions which present with low bone mass (*e.g.*, osteoporosis) in mammals is demonstrated by the activity of the parathyroid hormones in conventional assays, including *in vivo* assays, receptor binding assay, cyclic AMP assays and fracture healing assays.

[0033] PTS893 is an analogue of the endogenous parathyroid hormone, in which certain sites of chemical instability are eliminated within N-terminal parathyroid hormone fragments by making appropriate amino acid substitutions at particular residues which results in stable and biologically active human parathyroid hormone fragments. N-terminal fragments of human parathyroid hormones include hPTH(1-34)OH muteins and hPTH(1-38)OH muteins. PTS893 comprises at least the first 27 N-terminal amino acid units of parathyroid hormone. Preferred parathyroid hormone derivatives are those comprising at least one amino acid unit replaced in one or more of the following positions of the parathyroid hormone sequence: 8-11, 13, 16-19, 21, 22, 29 to 34, particularly 8-11, 16-19, 33 and/or 34. These compounds exhibit desirable bone-forming properties both *in vivo* and *in vitro* which are equal to or above the level of natural PTH and its N-terminal fragments. See, European patent EP 0 672 057; published PCT patent application WO 94/02510; Kneissel M *et al., Bone* 28: 237-50 (March 2001); Stewart AF *et al., J Bone Miner Res* 15(8): 1517-25 (August 2000); Thomsen JS *et al., Bone* 25(5):561-9 (November 1999).

[0034] *Kits*. The kits of the invention may contain a written product on or in the kit container. The written product describes how to use the reagents contained in the kit, *e.g.*, to determine whether a patient is responding effectively or can respond effectively to a compound for use in treating a condition for which calcitonin, parathyroid hormone, a parathyroid hormone analogue or a combination thereof is indicated. In several embodiments, the use of the reagents can be according to the methods of the invention. In one embodiment, the reagent is a gene chip for determining the gene expression of relevant genes.

[0035] The following EXAMPLE is presented in order to more fully illustrate the preferred embodiments of the invention. This EXAMPLE should in no way be construed as limiting the scope of the invention, as defined by the appended claims.

EXAMPLE

SALMON CALCITONIN AND PTS893, PHARMACOGENOMICS EXPLORATORY STUDY IN MONKEYS; MICROARRAY GENE EXPRESSION ANALYSIS

[0036] *Introduction and summary*. The purpose of this EXAMPLE was to evaluate the gene expression changes in cynomolgus monkeys following a two-week subcutaneous treatment with salmon calcitonin (sCT) at 50 μg/animal/day and PTS893 at 5 μg/animal/day to elucidate the mechanisms of action mediating their effects as well as the identification of biomarkers of therapeutic indications. This EXAMPLE is believed to be the first analysis that globally describes the molecular mechanisms of action of salmon calcitonin and a parathyroid hormone analogue by multi-organ-gene-profiling analysis in primates. This is also believed to be the first gene profiling analysis which describes the molecular mechanisms of action of hormonal-mediated bone remodelling by salmon calcitonin and PTS893.

[0037] In this EXAMPLE, salmon calcitonin and PTS893 were both found to have modulating effects on genes affecting the direct, autocrine, paracrine and endocrine regulation of the mesenchymal cell functions such as transforming growth factor betas (TGF-βs), insulin-like growth factors (IGFs), bone morphogenetic proteins (BMPs) and vascular endothelial growth factor (VEGF). Both compounds also regulate the synthesis and degradation of extracellular matrix components. Salmon calcitonin also regulates oestrogen receptor and steroidogenic factor, whereas PTS893 produced a strong up-regulation on

nuclear receptors of the steroid/thyroid receptor family. These data therefore support the role of calcitonin as an anabolic agent.

[0038] In addition, salmon calcitonin and PTS893 also influenced some aspects of the mineralization of the extracellular matrix, since changes in amelogenin, dentin and ectonucleotide pyrophosphatases were observed.

[0039] In addition, PTS893 showed an effect on mediating the paracrine activation of osteoclast differentiation and activity, through cytokine and RANK ligand.

[0040] No significant differences in gene expression profiling were attributable to the fact of administering salmon calcitonin and PTS893 in combination, with respect to the single therapy.

[0041] Thus, gene profiling analysis in this EXAMPLE allowed the reconstruction of the pathways involved in calcitonin and parathyroid hormone signal transduction, triggered by protein-G-linked-receptor stimulation and their influence on cell cycle, as indicated by the changes observed in cyclins.

[0042] *Animals*. A two-week subcutaneous treatment was carried out with salmon calcitonin (sCT), PTS893 or a combination of the two, each of which were dissolved in phosphate buffered saline (PBS) containing 9% autologous serum. Solvent was used as vehicle for the control group.

[0043] The animals used in this analysis were cynomolgus monkeys (*Macaca fascicularis*), supplied by Centre de Recherches Primatologiques, Port Louis, Mauritius. Two animals were used per group and sex. At the beginning of the treatment period, the animals were at least 24 months old, with a body weight of approximately 3 kg. Animals were kept under standard conditions for animal welfare. Animals were examined daily for mortality, food consumption and clinical observations. Body weight was recorded once per week. The dosages were 0 μg/animal/day (as the control), 50 μg/animal/day of salmon calcitonin and 5 μg/animal/day of PTS893.

[0044] As shown below, clinical observations and analysis, as well as the histopathological examinations performed in this EXAMPLE, showed that salmon calcitonin administered subcutaneously at a dose of 50 μg/animal/day was well tolerated by the cynomolgus monkeys.

[0045] *In vivo examinations.* No significant histopathological changes were observed. No relevant changes were observed other than a body weight decrease ranging from 8 to 12% in the salmon calcitonin group. A decrease in food consumption was also observed, although not always consistent with the decrease in body weight.

TABLE 1
Food Consumption – Males

Control

Day	-6	-5	-4	-3	-2	-1	1	2	3	4	5
Animal no. W62501	50	100	100	100	100	100	100	100	100	100	100
Animal no. W62502	50	100	100	100	100	100	100	100	100	100	25
Day	6	7	8	9	10	11	12	13	14		Avg.
Animal no. W62501	75	100	100	100	100	100	100	25			91.7
Animal no. W62502	100	75	75	100	100	100	75	100	50		91.7
Both animals											91.7

Salmon Calcitonin

Day	-6	-5	-4	-3	-2	-1	1	2	3	4	5
Animal no. W62503	50	75	50	75	100	75	75	25	50	100	100
Animal no. W62504	50	75	75	75	100	75	50	25	100	75	100
Day	6	7	8	9	10	11	12	13	14		Avg.
Animal no.W62503	75	100	100	100	100	75	75	25			70.8
Animal no.W62504	75	75	75	100	100	75	75	25	75		75.0
Both animals											72.9

PTS893

Day	-6	-5	-4	-3	-2	-1	1	2	3	4	5
Animal no. W62505	50	100	100	100	75	100	100	100	100	100	100
Animal no. W62506	50	100	100	100	100	100	100	100	100	100	100
Day	6	7	8	9	10	11	12	13	14		Avg.
Animal no. W62505	100	100	100	100	100	100	100	50	75		87.5
Animal no. W62506	100	100	100	100	100	100	100	100	100		91.7
Both animals											89.6

[0046] The animals to whom salmon calcitonin was administered presented with a decrease in body weight ranging between 8 to 12%, which can be attributed to a decrease in food consumption. An anorectic effect had previously been described for salmon calcitonin acting through amylin receptors Eiden S *et al., J. Physiol.* 541(pt3): 1041-1048 (2002); Lutz TA *et al., Peptides* 21 (2): 233-8 (2000). However, no signs of toxicity were observed here.

Hormonal and lipid changes observed in this EXAMPLE are most probably related to a consequent metabolic adaptation.

[0047] No relevant changes in electrocardiograms (ECG) or blood pressure were observed.

TABLE 2
Blood Pressure

Animal number	Sex	Compound administered	Week -1 (mm Hg)	Week 2 (mm Hg)	Difference (mm Hg)
W62501	Male	Control	121	98	-23
W62501	Male	Control	90	29	-61
W62502	Male	Control	86	107	21
W62502	Male	Control	26	34	8
W62503	Male	Salmon Calcitonin	135	99	-36
W62503	Male	Salmon Calcitonin	61	40	-21
W62504	Male	Salmon Calcitonin	102	79	-23
W62504	Male	Salmon Calcitonin	56	35	-21
W62505	Male	PTS893	76	87	11
W62505	Male	PTS893	18	22	4
W62506	Male	PTS893	106	101	-5
W62506	Male	PTS893	53	33	-20
W62551	Female	Control	96	76	-20
W62551	Female	Control	27	26	-1
W62552	Female	Control	102	93	-9
W62552	Female	Control	26	36	10
W62553	Female	Salmon Calcitonin	98	82	-16
W62553	Female	Salmon Calcitonin	50	25	-25
W62554	Female	Salmon Calcitonin	92	44	-48
W62554	Female	Salmon Calcitonin	26	30	4
W62555	Female	PTS893	92	70	-22
W62555	Female	PTS893	43	42	-1
W62556	Female	PTS893	78	87	9
W62556	Female	PTS893	24	28	4

[0048] *Blood sampling.* Animals were fasted overnight before blood collection but had free access to water. Blood samples were taken from a peripheral vein. Standard haematology and clinical chemistry analysis were performed once during pretest and at the end of the treatment period. Blood samples were collected from each animal at the same intervals as described for the clinical chemistry investigations. The serum samples were deep-frozen (approximately -80°C) until analyses for hormone determination.

[0049] *Clinical chemistry and hormone determinations.* A slight anaemia was observed in all animals of the study, including the controls. This was attributed to the repeated blood sampling and not considered to be relevant.

TABLE 3
Haematology – Males

Control

Animal no.			W62501			W62502	
Test	Units	d-6	d7	d13	d-6	d7	d13
WBC	G/l	10.0	11.1	12.9	6.1	11.2	6.3
RBC	T/l	7.3	6.5	6.4	6.8	6.5	6.2
HB	g/dl	12.9	11.9	11.7	13.1	12.3	11.9
PCV	l/l	0.44	0.40	0.44	0.42	0.41	0.41
MCV	fl	60	61	68	61	63	66
MCH	pg	17.8	18.2	18.1	19.3	19.0	19.0
MCHC	g/dl	29.8	29.6	26.8	31.5	30.1	28.9
PLAT	G/l	316	371	266	458	500	547
N	G/l	6.46	4.93	3.65	2.09	6.77	1.24
E	G/l	0.01	0.14	0.20	0.10	0.10	0.10
B	G/l	0.02	0.03	0.06	0.02	0.02	0.00
L	G/l	3.05	5.45	8.44	3.60	3.65	4.51
M	G/l	0.46	0.51	0.54	0.33	0.64	0.46

Salmon Calcitonin

Animal no.			W62503			W62504	
Test	Units	d-6	d7	d13	d-6	d7	d13
WBC	G/l	7.7	11.8	8.0	11.5	9.5	8.8
RBC	T/l	6.3	5.9	5.6	6.9	6.0	5.4
HB	g/dl	12.6	11.7	11.2	13.6	11.5	10.3
PCV	l/l	0.40	0.39	0.39	0.43	0.37	0.36
MCV	fl	64	66	70	62	62	67
MCH	pg	20.2	19.9	20.2	19.7	19.2	19.2
MCHC	g/dl	31.4	30.3	29.0	32.0	31.3	28.7
PLAT	G/l	351	396	302	247	330	389
N	G/l	3.36	4.11	1.90	3.93	3.31	3.04
E	G/l	0.02	0.10	0.13	0.16	0.09	0.01
B	G/l	0.02	0.04	0.03	0.08	0.04	0.03
L	G/l	4.00	6.79	5.38	6.55	5.57	4.92
M	G/l	0.30	0.73	0.57	0.76	0.45	0.76

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 3
Haematology – Males

PTS893

Animal no.			W62505			W62506	
Test	Units	d-6	d7	d13	d-6	d7	d13
WBC	G/l	10.4	8.4	8.8	9.1	15.0	11.9
RBC	T/l	7.6	6.4	6.8	6.5	5.9	5.8
HB	g/dl	13.6	11.3	11.7	13.2	11.9	11.8
PCV	l/l	0.43	0.38	0.43	0.40	0.40	0.41
MCV	fl	57	60	63	62	67	70
MCH	pg	18.0	17.7	17.3	20.4	20.2	20.3
MCHC	g/dl	31.5	29.3	27.5	33.1	30.2	29.2
PLAT	G/l	325	456	330	459	589	452
N	G/l	4.45	1.77	2.88	4.80	8.73	6.51
E	G/l	0.21	0.30	0.19	0.03	0.08	0.07
B	G/l	0.00	0.02	0.04	0.02	0.03	0.03
L	G/l	5.07	5.91	5.37	3.99	5.30	4.86
M	G/l	0.62	0.39	0.27	0.27	0.83	0.46

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 4
Haematology – Females

Control

Animal no.			W62551			W62552	
Test	Units	d-8	d7	d13	d-8	d7	d13
WBC	pg/ml	8.2	13.7	10.0	10.1	9.1	10.4
RBC	nmol/l	6.5	6.2	5.8	6.7	6.2	5.8
HB	pg/ml	12.8	11.8	11.3	13.1	11.7	11.4
PCV	mU/l	0.42	0.43	0.41	0.42	0.42	0.41
MCV	pg/ml	64	69	71	63	68	70
MCH	ng/ml	19.7	19.1	19.4	19.5	18.9	19.5
MCHC	pg/ml	30.6	27.7	27.4	30.9	27.7	27.9
PLAT	nmol/l	463	445	468	286	292	275
N	nmol/l	4.45	5.86	3.53	6.69	3.13	4.23
E	mUI/l	0.03	0.13	0.12	0.01	0.15	0.19
B	pg/ml	0.03	0.07	0.04	0.02	0.03	0.03
L	pg/ml	3.40	7.09	5.91	3.14	5.39	5.34
M	nmol/l	0.27	0.51	0.39	0.25	0.39	0.59

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

TABLE 4
Haematology – Females

Salmon Calcitonin

Animal no.			W62553			W62554	
Test	Units	d-8	d7	d13	d-8	d7	d13
WBC	pg/ml	7.0	9.5	12.0	8.3	17.0	13.3
RBC	nmol/l	6.5	6.2	5.2	7.0	6.6	5.7
HB	pg/ml	12.3	11.5	10.1	13.8	12.7	11.0
PCV	mU/l	0.40	0.40	0.33	0.45	0.44	0.37
MCV	pg/ml	61	64	64	65	68	65
MCH	ng/ml	19.1	18.6	19.5	19.8	19.4	19.5
MCHC	pg/ml	31.2	29.0	30.3	30.6	28.7	29.9
PLAT	nmol/l	549	594	451	304	356	229
N	nmol/l	3.45	3.83	5.41	3.13	9.82	6.16
E	mUI/l	0.03	0.36	0.73	0.03	0.04	0.06
B	pg/ml	0.02	0.03	0.03	0.01	0.07	0.05
L	pg/ml	3.26	4.61	5.18	4.79	6.21	6.58
M	nmol/l	0.25	0.63	0.69	0.30	0.82	0.39

PTS893

Animal no.			W62555			W62556	
Test	Units	d-8	d7	d13	d-8	d7	d13
WBC	pg/ml	10.1	18.4	13.2	14.3	12.3	10.1
RBC	nmol/l	6.9	6.2	5.9	6.7	6.4	5.9
HB	pg/ml	13.4	11.7	11.3	12.9	12.1	11.3
PCV	mU/l	0.44	0.41	0.40	0.43	0.43	0.39
MCV	pg/ml	63	67	67	64	68	66
MCH	ng/ml	19.3	18.9	19.3	19.3	19.0	19.2
MCHC	pg/ml	30.6	28.2	28.6	30.2	28.1	29.2
PLAT	nmol/l	501	525	496	213	382	309
N	nmol/l	5.34	10.8	6.36	9.05	5.49	4.18
E	mUI/l	0.00	0.12	0.21	0.26	0.49	0.29
B	pg/ml	0.00	0.06	0.03	0.03	0.04	0.04
L	pg/ml	3.92	6.29	5.81	4.40	5.87	5.21
M	nmol/l	0.80	1.12	0.82	0.54	0.44	0.37

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

[0050] Among the standard clinical chemistry tests performed, slight to moderate decreases in phosphorus and/or magnesium and a moderate to marked decrease in triglycerides were seen in the groups administered salmon calcitonin and PTS893.

TABLE 5
Clinical Chemistry – Males

Control

Animal no.			W62501			W62502	
Test	Units	d-6	d7	d13	d-6	d7	d13
Na+	mmol/l	154	151	153	152	153	148
K+	mmol/l	4.05	5.31	4.26	4.09	4.05	4.51
Cl-	mmol/l	109	113	108	107	110	111
Ca++	mmol/l	2.57	2.47	2.69	2.72	2.52	2.75
I.PHOS	mmol/l	2.21	1.93	2.76	1.88	1.69	1.99
Mg++	mmol/l	1.09	0.91	0.95	0.88	0.79	1.14
GLUC	mmol/l	3.85	4.51	4.68	3.44	5.30	6.13
UREA	mmol/l	9.7	4.9	5.0	7.6	6.1	5.2
CREAT	µmol/l	85	60	75	65	55	57
TOT.BIL.	µmol/l	6.0	2.0	2.0	7.0	3.0	4.0
PROT	g/l	89	80	88	90	83	85
A/G		1.89	1.57	1.45	1.62	1.53	1.50
CHOL	mmol/l	3.30	3.20	3.50	3.30	3.40	3.10
HDL-CHOL	mmol/l	1.49	1.45	1.70	1.54	1.45	1.49
LDL-CHOL	mmol/l	1.63	1.62	1.84	1.56	1.93	1.49
TRIG	mmol/l	0.94	0.36	0.43	0.65	0.36	0.45
ALP	IU/l	1559	1241	1313	1463	1423	1493
BAP-E	IU/l	543	439	457	452	476	464
ASAT	IU/l	22	22	25	30	26	26
ALAT	IU/l	22	32	30	29	41	37
CK	IU/l	150	45	127	74	67	102
LDH	IU/l	392	585	549	421	518	592
GGT	IU/l	128	92	111	89	71	75
ALB	%	65	61	59	62	61	60
A1-GLOB	%	1.90	2.70	2.50	1.90	2.10	2.30
A2-GLOB	%	7.60	8.30	7.90	8.20	8.90	8.50
B-GLOB	%	16	18	19	18	19	19
G-GLOB	%	9.2	9.9	10.9	9.6	9.3	10.2
ALB	g/l	58	49	52	56	50	51
A1-GLOB	g/l	1.70	2.20	2.20	1.70	1.70	2.00
A2-GLOB	g/l	6.80	6.60	7.00	7.40	7.40	7.20
B-GLOB	g/l	14	14	17	17	16	16
G-GLOB	g/l	8.2	7.9	9.6	8.6	7.7	8.7

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 5
Clinical Chemistry – Males

Salmon Calcitonin

Animal no.			W62503			W62504	
Test	Units	d-6	d7	d13	d-6	d7	d13
Na+	mmol/l	151	145	148	154	142	144
K+	mmol/l	4.24	4.90	4.34	4.85	5.15	4.48
Cl-	mmol/l	107	104	104	113	106	101
Ca++	mmol/l	2.66	2.68	2.91	2.71	2.54	2.73
I.PHOS	mmol/l	2.05	1.67	2.06	2.10	1.73	1.94
Mg++	mmol/l	0.97	0.68	0.73	0.99	0.71	0.72
GLUC	mmol/l	3.57	3.58	4.29	3.70	4.98	6.19
UREA	mmol/l	7.9	1.3	2.9	6.6	3.3	2.9
CREAT	µmol/l	78	57	62	64	50	56
TOT.BIL.	µmol/l	5.0	2.0	1.0	3.0	2.0	2.0
PROT	g/l	87	82	87	91	83	89
A/G		1.76	1.68	1.42	1.42	1.26	1.05
CHOL	mmol/l	3.30	3.60	3.70	3.80	3.90	3.40
HDL-CHOL	mmol/l	1.49	2.09	2.44	1.46	1.48	1.39
LDL-CHOL	mmol/l	1.21	1.28	1.26	1.87	2.51	1.83
TRIG	mmol/l	0.96	0.24	0.27	0.92	0.22	0.68
ALP	IU/l	1488	1023	1226	857	587	626
BAP-E	IU/l	508	363	302	311	188	180
ASAT	IU/l	28	31	28	24	17	24
ALAT	IU/l	38	39	43	48	24	31
CK	IU/l	124	56	119	75	45	173
LDH	IU/l	439	400	427	356	384	519
GGT	IU/l	105	80	75	121	75	69
ALB	%	64	63	59	59	56	51
A1-GLOB	%	1.60	2.00	2.40	1.90	2.80	3.60
A2-GLOB	%	8.00	8.80	8.80	8.70	8.70	7.80
B-GLOB	%	18	18	20	19	21	24
G-GLOB	%	8.3	8.5	9.7	12.0	12.1	13.6
ALB	g/l	56	51	51	54	46	46
A1-GLOB	g/l	1.40	1.60	2.10	1.70	2.30	3.20
A2-GLOB	g/l	7.00	7.20	7.70	7.90	7.20	6.90
B-GLOB	g/l	16	15	18	17	17	21
G-GLOB	g/l	7.2	7.0	8.4	10.9	10.0	12.1

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 5
Clinical Chemistry – Males

PTS893

Animal no.			W62505			W62506	
Test	Units	d-6	d7	d13	d-6	d7	d13
Na+	mmol/l	151	151	152	151	149	149
K+	mmol/l	5.13	4.00	4.27	4.72	4.76	4.12
Cl-	mmol/l	110	107	110	112	106	106
Ca++	mmol/l	2.81	2.39	2.59	2.64	2.45	2.51
I.PHOS	mmol/l	2.59	1.68	2.22	2.12	1.12	1.77
Mg++	mmol/l	1.04	0.71	0.77	0.97	0.70	0.76
GLUC	mmol/l	5.09	4.76	5.42	3.88	5.26	4.96
UREA	mmol/l	11.6	3.7	6.4	15.0	4.9	5.8
CREAT	µmol/l	86	66	79	77	63	70
TOT.BIL.	µmol/l	5.0	2.0	1.0	7.0	2.0	1.0
PROT	g/l	81	74	81	88	86	89
A/G		1.89	1.70	1.76	1.58	1.28	1.40
CHOL	mmol/l	3.20	3.30	3.10	2.50	2.50	2.60
HDL-CHOL	mmol/l	1.49	1.49	1.61	1.24	1.25	1.38
LDL-CHOL	mmol/l	1.39	1.73	1.51	1.27	1.22	1.38
TRIG	mmol/l	0.96	0.30	0.63	0.49	0.39	0.35
ALP	IU/l	1703	1494	1768	1414	1363	1486
BAP-E	IU/l	523	532	564	445	423	497
ASAT	IU/l	24	18	24	25	27	29
ALAT	IU/l	32	30	27	23	19	20
CK	IU/l	111	82	148	86	73	125
LDH	IU/l	367	400	528	354	432	464
GGT	IU/l	133	99	105	112	85	91
ALB	%	66	63	64	61	56	59
A1-GLOB	%	2.20	2.80	2.60	2.40	3.60	2.80
A2-GLOB	%	8.80	8.90	8.70	7.30	8.30	7.50
B-GLOB	%	17	18	19	19	22	20
G-GLOB	%	6.9	6.9	6.3	9.8	10.5	10.9
ALB	g/l	53	47	52	54	48	52
A1-GLOB	g/l	1.80	2.10	2.10	2.10	3.10	2.50
A2-GLOB	g/l	7.10	6.60	7.10	6.40	7.10	6.70
B-GLOB	g/l	14	14	15	17	19	18
G-GLOB	g/l	5.6	5.1	5.1	8.6	9.0	9.7

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 6
Clinical Chemistry – Females

Control

Animal no.			W62551			W62552	
Test	Units	d-8	d7	d13	d-8	d7	d13
Na+	mmol/l	152	148	155	148	150	148
K+	mmol/l	4.16	4.23	4.92	3.82	4.11	5.27
Cl-	mmol/l	110	105	111	109	106	108
Ca++	mmol/l	2.64	2.61	2.61	2.48	2.44	1.80
I.PHOS	mmol/l	1.98	2.61	2.28	1.84	1.98	1.84
Mg++	mmol/l	1.00	0.97	1.03	0.88	0.84	0.31
GLUC	mmol/l	3.65	8.39	3.86	2.79	3.86	3.60
UREA	mmol/l	11.0	8.3	8.2	11.3	6.9	6.3
CREAT	µmol/l	73	77	62	67	60	50
TOT.BIL.	µmol/l	4.00	2.00	3.00	5.00	1.00	2.00
PROT	g/l	85	80	80	83	83	77
A/G		1.77	1.67	1.55	1.68	1.39	1.27
CHOL	mmol/l	3.20	2.80	3.00	3.70	3.40	3.50
HDL-CHOL	mmol/l	1.63	1.44	1.49	1.75	1.82	1.80
LDL-CHOL	mmol/l	1.55	1.25	1.90	1.57	1.28	1.66
TRIG	mmol/l	0.64	0.54	0.57	0.83	0.48	0.50
ALP	IU/l	1037	1088	1187	1332	1298	1182
BAP-E	IU/l	310	369	346	432	419	379
ASAT	IU/l	27	33	31	21	22	23
ALAT	IU/l	44	52	46	16	19	20
CK	IU/l	69	169	81	83	68	87
LDH	IU/l	420	520	481	474	471	516
GGT	IU/l	104	95	102	84	67	66
ALB	%	64	63	61	63	58	56
A1-GLOB	%	1.90	2.60	3.40	2.00	2.60	3.50
A2-GLOB	%	8.00	7.60	7.70	7.00	8.10	7.70
B-GLOB	%	17	18	18	15	18	18
G-GLOB	%	9.4	9.2	9.9	12.9	13.2	14.8
ALB	g/l	54	50	49	52	48	43
A1-GLOB	g/l	1.60	2.10	2.70	1.70	2.20	2.70
A2-GLOB	g/l	6.80	6.10	6.20	5.80	6.70	5.90
B-GLOB	g/l	14	14	15	13	15	14
G-GLOB	g/l	8.0	7.4	7.9	10.7	11.0	11.4

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

TABLE 6
Clinical Chemistry – Females

Salmon Calcitonin

Animal no.		W62553			W62554		
Test	Units	d-8	d7	d13	d-8	d7	d13
Na+	mmol/l	145	147	147	145	143	147
K+	mmol/l	3.51	3.73	4.62	3.89	4.07	4.95
Cl-	mmol/l	106	104	107	100	96	107
Ca++	mmol/l	2.62	2.77	2.57	2.73	2.91	2.68
I.PHOS	mmol/l	1.62	1.48	1.81	1.97	1.75	1.83
Mg++	mmol/l	0.87	0.63	0.76	0.91	0.77	0.80
GLUC	mmol/l	3.84	4.88	4.98	4.11	5.31	4.04
UREA	mmol/l	10.3	6.6	5.0	10.0	6.3	5.9
CREAT	µmol/l	81	71	61	88	77	65
TOT.BIL.	µmol/l	3.00	2.00	2.00	6.00	5.00	2.00
PROT	g/l	88	90	80	91	95	83
A/G		1.46	1.45	1.30	1.48	1.42	1.26
CHOL	mmol/l	2.70	2.80	2.20	3.30	4.00	3.00
HDL-CHOL	mmol/l	1.04	1.11	0.96	1.46	1.99	1.66
LDL-CHOL	mmol/l	1.61	1.51	1.46	1.13	1.93	1.42
TRIG	mmol/l	0.79	0.25	0.39	0.88	0.30	0.38
ALP	IU/l	1197	965	842	1132	877	890
BAP-E	IU/l	416	326	304	344	325	294
ASAT	IU/l	24	21	25	20	18	20
ALAT	IU/l	21	24	19	19	14	19
CK	IU/l	99	72	107	76	64	77
LDH	IU/l	286	423	429	319	372	363
GGT	IU/l	88	63	54	82	72	62
ALB	%	59	59	57	60	59	56
A1-GLOB	%	2.70	2.70	3.10	2.20	2.20	3.10
A2-GLOB	%	6.50	6.10	6.80	8.00	7.70	7.80
B-GLOB	%	21	23	21	15	17	17
G-GLOB	%	10.8	8.6	12.4	14.9	14.6	16.3
ALB	g/l	52	54	45	54	56	46
A1-GLOB	g/l	2.40	2.40	2.50	2.00	2.10	2.60
A2-GLOB	g/l	5.70	5.50	5.40	7.30	7.30	6.50
B-GLOB	g/l	18	21	17	14	16	14
G-GLOB	g/l	9.5	7.7	9.9	13.6	13.9	13.5

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

TABLE 6
Clinical Chemistry – Females

PTS893

Animal no.			W62555			W62556	
Test	Units	d-8	d7	d13	d-8	d7	d13
Na+	mmol/l	153	151	152	150	148	149
K+	mmol/l	4.82	4.54	4.63	3.85	3.81	4.31
Cl-	mmol/l	107	109	111	108	107	114
Ca++	mmol/l	2.77	2.61	2.20	2.64	2.62	2.35
I.PHOS	mmol/l	2.11	1.31	1.51	2.10	1.60	1.50
Mg++	mmol/l	0.96	0.65	0.59	0.90	0.74	0.66
GLUC	mmol/l	3.57	4.18	3.59	3.22	4.45	3.52
UREA	mmol/l	8.2	8.7	6.3	8.4	6.6	6.8
CREAT	µmol/l	77	62	58	68	63	58
TOT.BIL.	µmol/l	5.00	1.00	2.00	5.00	2.00	2.00
PROT	g/l	89	87	78	84	83	76
A/G		1.64	1.62	1.65	1.84	1.78	1.50
CHOL	mmol/l	2.90	2.70	2.80	2.70	2.40	2.70
HDL-CHOL	mmol/l	1.31	1.48	1.51	1.12	0.99	1.25
LDL-CHOL	mmol/l	1.69	1.12	1.71	1.62	1.28	1.58
TRIG	mmol/l	0.59	0.27	0.25	0.67	0.34	0.47
ALP	IU/l	1535	1223	1332	1638	1307	1313
BAP-E	IU/l	457	350	426	456	390	400
ASAT	IU/l	23	18	25	24	20	25
ALAT	IU/l	35	25	32	33	19	21
CK	IU/l	84	65	175	63	144	172
LDH	IU/l	468	465	557	309	313	358
GGT	IU/l	85	71	70	103	85	83
ALB	%	62	62	62	65	64	60
A1-GLOB	%	2.30	2.50	2.50	1.90	2.10	2.70
A2-GLOB	%	7.50	8.00	8.30	7.50	7.50	8.10
B-GLOB	%	18	19	18	17	17	20
G-GLOB	%	9.7	8.4	8.7	8.8	9.1	8.7
ALB	g/l	55	54	49	55	53	46
A1-GLOB	g/l	2.10	2.20	2.00	1.60	1.70	2.10
A2-GLOB	g/l	6.70	7.00	6.50	6.30	6.20	6.20
B-GLOB	g/l	16	17	14	14	14	16
G-GLOB	g/l	8.6	7.3	6.8	7.4	7.6	6.6

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

[0051] No relevant changes were observed in the standard urinalysis tests performed.

TABLE 7
Urinary analysis – Males

Control

Animal no.			W62501			W62502	
Test	Units	-6	-5	13	-6	-5	13
VOLUME	ml	15	10	77	22	130	30
CREAT	µmol/l	18000	17000	5460	7920	2480	5160
NTx	nM BCE	-	9954	3425	-	11979	3167
CTx	µg/l	-	21592	6810	-	27169	5323
D-PYR	nmol/l	-	2345	1110	-	2904	1461
LDH	IU/L	6.0		nd	8.0		8.0
NAG	IU/l	3.5		1.5	3.2		1.6
Na+	mmol/l	163		43	87		77
K+	mmol/l	258		67	125		75
Cl-	mmol/l	132		43	52		59
Ca2+	mmol/l	5.15		16.80	15.95		15.50
I.PHOS	mmol/l	11.10		1.05	11.30		8.90
Mg2+	mmol/l	2.75		7.50	7.85		6.25
Na/Crea	mM/mM	9.10		7.90	11.00		14.90
K/Crea	mM/mM	14.30		12.20	15.80		14.50
Cl/Crea	mM/mM	7.40		7.90	6.50		11.40
Ca/Crea	mM/mM	0.29		3.08	2.01		3.00
Pho/Crea	mM/mM	0.62		0.19	1.43		1.73
Mg/Crea	mM/mM	0.20		1.40	1.00		1.20
LDH/crea	IU/mM	0.33		nd	1.01		1.55
NAG/crea	IU/mM	0.19		0.28	0.40		0.31
NTx/Crea	nME/mM		586	627		4830	614
CTx/Crea	µg/µm.		1270	1247		10955	1032
Pyr/Crea	nM/mM		138	203		1171	283

d-6, d-5 and d13 indicate day -6, day -5 and day 13 relative to the starting day of dosing

TABLE 7

Urinary analysis – Males

Salmon Calcitonin

Animal no.			W62503			W62504	
Test	Units	-6	-5	13	-6	-5	13
VOLUME	ml	62	38	68	37	10	54
CREAT	µmol/l	4300	7840	4620	13600	17360	4400
NTx	nM BCE	-	6023	5186	-	16067	3790
CTx	µg/l	-	11618	10088	-	26370	6130
D-PYR	nmol/l	-	1733	1083	-	5113	1476
LDH	IU/L	9.0		7.0	13.0		17.0
NAG	IU/l	2.7		1.4	4.2		7.2
Na+	mmol/l	22		14	119		15
K+	mmol/l	65		78	134		76
Cl-	mmol/l	10		55	64		68
Ca2+	mmol/l	0.90		18.25	3.70		23.40
I.PHOS	mmol/l	4.35		2.50	5.33		3.00
Mg2+	mmol/l	1.40		7.05	7.55		9.80
Na/Crea	mM/mM	5.20		3.10	8.70		3.40
K/Crea	mM/mM	15.10		16.90	9.90		17.20
Cl/Crea	mM/mM	2.20		11.80	4.70		15.30
Ca/Crea	mM/mM	0.21		3.95	0.27		5.32
Pho/Crea	mM/mM	1.01		0.54	0.39		0.68
Mg/Crea	mM/mM	0.30		1.50	0.60		2.20
LDH/crea	IU/mM	2.09		1.52	0.96		3.86
NAG/crea	IU/mM	0.63		0.30	0.31		1.64
NTx/Crea	nME/mM		768	1123		926	861
CTx/Crea	µg/µm.		1482	2184		1519	1393
Pyr/Crea	nM/mM		221	234		295	336

d-6, d-5 and d13 indicate day -6, day -5 and day 13 relative to the starting day of dosing

TABLE 7
Urinary analysis – Males

PTS893

Animal no.			W62505			W62506	
Test	Units	-6	-5	13	-6	-5	13
VOLUME	ml	14	14	48	58	34	130
CREAT	µmol/l	16160	16160	7840	9940	16120	3840
NTx	nM BCE	-	5403	4871	-	8757	2102
CTx	µg/l	-	11865	9365	-	20108	3705
D-PYR	nmol/l	-	1660	1676	-	2278	782
LDH	IU/L	7.0		14.0	9.0		19.0
NAG	IU/l	23.4		2.9	7.1		2.6
Na+	mmol/l	174		111	59		35
K+	mmol/l	86		107	125		69
Cl-	mmol/l	22		117	50		48
Ca2+	mmol/l	5.10		7.55	3.50		13.10
I.PHOS	mmol/l	74.40		0.10	3.86		0.17
Mg2+	mmol/l	11.25		8.70	2.95		5.25
Na/Crea	mM/mM	10.80		14.10	6.00		9.10
K/Crea	mM/mM	5.30		13.60	12.60		17.90
Cl/Crea	mM/mM	1.40		15.00	5.00		12.60
Ca/Crea	mM/mM	0.32		0.96	0.35		3.41
Pho/Crea	mM/mM	4.60		0.01	0.39		0.04
Mg/Crea	mM/mM	0.70		1.10	0.30		1.40
LDH/crea	IU/mM	0.43		1.79	0.91		4.95
NAG/crea	IU/mM	1.45		0.37	0.71		0.68
NTx/Crea	nME/mM		334	621		543	547
CTx/Crea	µg/µm.		734	1195		1247	965
Pyr/Crea	nM/mM		103	214		141	204

d-6, d-5 and d13 indicate day -6, day -5 and day 13 relative to the starting day of dosing

TABLE 8
Urinary analysis – Females

Control

Animal no.			W62551			W62552	
Test	Units	-8	-7	13	-8	-7	13
VOLUME	ml	21	21	43	18	53	53
CREAT	µmol/l	16420	16420	9560	14300	6700	5380
NTx	nM BCE	-	9248	7824	-	5053	4695
CTx	µg/l	-	19280	17916	-	12014	10557
D-PYR	nmol/l	-	2500	2748	-	1397	2159
LDH	IU/L	10.0		15.0	9.0		25.0
NAG	IU/l	19.2		4.2	10.3		3.5
Na+	mmol/l	110		44	140		64
K+	mmol/l	82		122	124		87
Cl-	mmol/l	24		73	72		56
Ca2+	mmol/l	2.90		16.10	11.90		19.50
I.PHOS	mmol/l	88.2		7.7	20.3		3.5
Mg2+	mmol/l	2.35		7.20	9.00		5.45
Na/Crea	mM/mM	6.70		4.60	9.80		11.90
K/Crea	mM/mM	5.00		12.80	8.70		16.20
Cl/Crea	mM/mM	1.50		7.60	5.10		10.50
Ca/Crea	mM/mM	0.18		1.68	0.83		3.63
Pho/Crea	mM/mM	5.37		0.81	1.42		0.64
Mg/Crea	mM/mM	0.10		0.80	0.60		1.00
LDH/crea	IU/mM	0.61		1.57	0.63		4.65
NAG/crea	IU/mM	1.17		0.44	0.72		0.65
NTx/Crea	nME/mM		563	818		754	873
CTx/Crea	µg/µm.		1174	1874		1793	1962
Pyr/Crea	nM/mM		152	288		209	401

d-8, d-7 and d13 indicate day -8, day -7 and day 13 relative to the starting day of dosing

TABLE 8
Urinary analysis – Females

Salmon Calcitonin

Animal no.			W62553			W62554	
Test	Units	-8	-7	13	-8	-7	13
VOLUME	ml	11	58	67	32	14	49
CREAT	µmol/l	10780	6920	4800	11260	13380	4200
NTx	nM BCE	-	4624	3465	-	7393	2812
CTx	µg/l	-	6983	5392	-	13411	5631
D-PYR	nmol/l	-	2762	1644	-	2016	1110
LDH	IU/L	14.0		6.0	6.0		36.0
NAG	IU/l	10.2		2.8	1.2		2.7
Na+	mmol/l	98		40	156		32
K+	mmol/l	104		53	172		57
Cl-	mmol/l	31		63	156		65
Ca2+	mmol/l	3.00		17.55	3.50		12.70
I.PHOS	mmol/l	25.4		5.1	10.8		5.8
Mg2+	mmol/l	3.35		5.40	3.80		4.85
Na/Crea	mM/mM	9.10		8.30	13.90		7.60
K/Crea	mM/mM	9.60		11.10	15.20		13.50
Cl/Crea	mM/mM	2.90		13.20	13.80		15.40
Ca/Crea	mM/mM	0.28		3.66	0.31		3.02
Pho/Crea	mM/mM	2.35		1.05	0.96		1.38
Mg/Crea	mM/mM	0.30		1.10	0.30		1.20
LDH/crea	IU/mM	1.30		1.25	0.53		8.57
NAG/crea	IU/mM	0.95		0.58	0.11		0.64
NTx/Crea	nME/mM		668	722		553	670
CTx/Crea	µg/µm.		1009	1123		1002	1341
Pyr/Crea	nM/mM		399	343		151	264

d-8, d-7 and d13 indicate day -8, day -7 and day 13 relative to the starting day of dosing

TABLE 8
Urinary analysis – Females

PTS893

Animal no.			W62555			W62556	
Test	Units	-8	-7	13	-8	-7	13
VOLUME	ml	14	15	52	39	69	42
CREAT	µmol/l	19160	18240	5620	14060	7600	8060
NTx	nM BCE	-	10499	2514	-	4818	5679
CTx	µg/l	-	21919	3813	-	8877	11236
D-PYR	nmol/l	-	2963	1356	-	1377	2036
LDH	IU/L	11.0		10.0	18.0		9.0
NAG	IU/l	0.5		1.2	5.9		5.1
Na+	mmol/l	145		71	118		146
K+	mmol/l	302		150	164		70
Cl-	mmol/l	119		101	53		133
Ca2+	mmol/l	11.50		20.05	6.60		12.35
I.PHOS	mmol/l	0.2		0.1	7.6		2.9
Mg2+	mmol/l	7.35		6.90	4.00		5.90
Na/Crea	mM/mM	7.60		12.60	8.40		18.10
K/Crea	mM/mM	15.80		26.80	11.70		8.60
Cl/Crea	mM/mM	6.20		18.00	3.70		16.50
Ca/Crea	mM/mM	0.60		3.57	0.47		1.53
Pho/Crea	mM/mM	0.01		0.02	0.54		0.36
Mg/Crea	mM/mM	0.40		1.20	0.30		0.70
LDH/crea	IU/mM	0.57		1.78	1.28		1.12
NAG/crea	IU/mM	0.03		0.21	0.42		0.63
NTx/Crea	nME/mM		576	447		634	705
CTx/Crea	µg/µm.		1202	679		1168	1394
Pyr/Crea	nM/mM		163	241		181	253

d-8, d-7 and d13 indicate day -8, day -7 and day 13 relative to the starting day of dosing

[0052] The salmon calcitonin group presented with moderate decreases in serum somatomedin (S.MED, see TABLES 9 and 10).

TABLE 9
Hormones – Males

Control

Animal no.			W62501			W62502	
Test	Units	d-6	d7	d13	d-6	d7	d13
ACTH	pg/ml	91	63	87	117	136	150
CORTISOL	nmol/l	2183	1415	1328	1378	1020	1348
ALDOST	pg/ml	316	433	484	501	644	622
INSULIN	mU/l	26.0	33.0	37.0	12.0	30.0	9.0
GLUCAG	pg/ml	791	486	704	577	353	585
C-PEPTI	ng/ml	n/a	5.20	5.50	n/a	3.60	1.60
GASTRIN	pg/ml	n/a	105	93	n/a	147	148
T3	nmol/l	1.34	2.61	2.94	2.19	2.73	2.50
T4	nmol/l	56	61	44	57	68	48
TSH	mUI/l	0.17	0.18	0.42	0.00	0.05	0.04
IPH	pg/ml	103	75	108	174	173	155
CT	pg/ml	5.9	4.6	4.8	16.4	15.0	13.1
VD25-H	nmol/l	49	47	54	76	71	58
VD1-25dh	pmol/l	n/a	-	-	n/a	-	-
OSTEO	ng/ml	n/a	26	34	n/a	41	40
CTx	nmol/l	10	15	20	17	19	20
ICTP	ng/ml	18	13	19	26	16	15
PICP	ng/ml	n/a	311	395	n/a	610	495
G.H.	ng/ml	13.8	7.0	16.2	15.2	3.6	17.2
S.STA	pg/ml	n/a	-	-	n/a	-	-
S.MED	**ng/ml**	**n/a**	**888**	**1185**	**n/a**	**793**	**689**
PROLACT	ng/ml	0.0	3.3	3.6	21.6	22.5	22.5
TESTO	nmol/l	10.5	8.4	n. s.	7.9	4.7	n. s.
ESTR	nmol/l	n/a	n/a	n/a	n/a	n/a	n/a
PROG	pmol/l	n/a	n/a	n/a	n/a	n/a	n/a

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 9
Hormones – Males

Salmon Calcitonin

Animal no.			W62503			W62504	
Test	Units	d-6	d7	d13	d-6	d7	d13
ACTH	pg/ml	98	87	87	115	78	73
CORTISOL	nmol/l	2316	979	1611	1578	1523	1709
ALDOST	pg/ml	983	1058	819	465	987	977
INSULIN	mU/l	13.0	14.0	17.0	4.0	10.0	22.0
GLUCAG	pg/ml	905	247	428	869	218	503
C-PEPTI	ng/ml	n/a	1.70	1.80	n/a	1.20	2.30
GASTRIN	pg/ml	n/a	83	88	n/a	128	136
T3	nmol/l	1.06	2.35	2.51	1.48	1.65	1.90
T4	nmol/l	53	64	47	62	79	65
TSH	mUI/l	0.99	1.12	1.03	0.14	0.41	0.40
IPH	pg/ml	213	75	78	99	62	71
CT	pg/ml	6.7	4.0	2.4	5.1	2.5	4.9
VD25-H	nmol/l	63	50	49	62	44	45
VD1-25dh	pmol/l	n/a	-	-	n/a	-	-
OSTEO	ng/ml	n/a	33	41	n/a	27	30
CTx	nmol/l	12	26	38	18	22	24
ICTP	ng/ml	21	15	15	22	21	20
PICP	ng/ml	n/a	284	363	n/a	361	439
G.H.	ng/ml	11.5	1.7	16.2	14.6	13.6	15.7
S.STA	pg/ml	n/a	-	-	n/a	-	-
S.MED	ng/ml	n/a	268	332	n/a	307	384
PROLACT	ng/ml	8.1	8.6	4.6	0.0	0.0	6.6
TESTO	nmol/l	8.5	3.6	n. s.	9.5	7.3	n. s.
ESTR	nmol/l	n/a	n/a	n/a	n/a	n/a	n/a
PROG	pmol/l	n/a	n/a	n/a	n/a	n/a	n/a

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 9
Hormones – Males

PTS893

Animal no.			W62505			W62506	
Test	Units	d-6	d7	d13	d-6	d7	d13
ACTH	pg/ml	96	101	83	115	88	91
CORTISOL	nmol/l	1662	1156	1299	1506	1432	1212
ALDOST	pg/ml	265	380	592	141	471	651
INSULIN	mU/l	16.0	22.0	14.0	12.0	38.0	10.0
GLUCAG	pg/ml	858	656	786	694	497	739
C-PEPTI	ng/ml	n/a	2.90	2.10	n/a	4.40	2.40
GASTRIN	pg/ml	n/a	84	78	n/a	98	94
T3	nmol/l	2.48	3.47	3.55	1.38	2.76	2.43
T4	nmol/l	84	90	68	59	80	56
TSH	mUI/l	0.22	0.40	0.15	0.00	0.07	0.03
IPH	pg/ml	123	96	78	71	62	55
CT	pg/ml	6.1	4.0	4.6	10.4	7.8	7.6
VD25-H	nmol/l	77	62	50	88	62	50
VD1-25dh	pmol/l	n/a	-	-	n/a	-	-
OSTEO	ng/ml	n/a	43	55	n/a	32	42
CTx	nmol/l	19	20	31	12	12	16
ICTP	ng/ml	28	23	22	18	16	18
PICP	ng/ml	n/a	420	500	n/a	774	706
G.H.	ng/ml	13.4	15.8	12.1	8.5	11.6	14.0
S.STA	pg/ml	n/a	-	-	n/a	-	-
S.MED	ng/ml	n/a	749	914	n/a	828	867
PROLACT	ng/ml	7.1	15.7	7.5	7.5	5.5	2.2
TESTO	nmol/l	11.8	10.5	n. s.	5.3	3.7	n. s.
ESTR	nmol/l	n/a	n/a	n/a	n/a	n/a	n/a
PROG	pmol/l	n/a	n/a	n/a	n/a	n/a	n/a

d-6, d7 and d13 indicate day -6, day 7 and day 13 relative to the starting day of dosing

TABLE 10
Hormones – Females

Control

Animal no.			W62551			W62552	
Test	Units	d-8	d7	d13	d-8	d7	d13
ACTH	pg/ml	146	276	121	58	60	101
CORTISOL	nmol/l	1983	1546	827	1894	837	818
ALDOST	pg/ml	244	953	312	149	90	199
INSULIN	mU/l	8.0	12.0	7.0	2.0	29.0	21.0
GLUCAG	pg/ml	729	779	583	818	507	514
C-PEPTI	ng/ml	n/a	2.40	1.40	n/a	3.30	2.30
GASTRIN	pg/ml	n/a	84	102	n/a	90	92
T3	nmol/l	2.22	2.95	3.40	2.04	3.09	3.23
T4	nmol/l	78	67	59	51	50	49
TSH	mUI/l	0.14	0.27	0.49	0.15	0.54	0.50
IPH	pg/ml	155	149	129	145	129	112
CT	pg/ml	4.70	3.90	4.10	11.50	11.60	11.20
VD25-H	nmol/l	64	59	51	80	78	70
VD1-25dh	pmol/l	n/a	-	-	n/a	-	-
OSTEO	ng/ml	n/a	37	39	n/a	34	39
CTx	nmol/l	11	26	28	12	16	20
ICTP	ng/ml	21	23	22	19	16	15
PICP	ng/ml	n/a	864	503	n/a	339	298
G.H.	ng/ml	8.5	13.4	1.7	7.0	12.0	4.5
S.STA	pg/ml	n/a	-	-	n/a	-	-
S.MED	ng/ml	n/a	696	839	n/a	1173	1527
PROLACT	ng/ml	4.30	8.30	5.90	2.90	0.00	0.00
TESTO	nmol/l						
ESTR	nmol/l	58	64	61	48	45	60
PROG	pmol/l	3.40	3.50	1.70	2.70	1.10	1.40

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

TABLE 10
Hormones – Females

Salmon Calcitonin

Animal no.			W62553			W62554	
Test	Units	d-8	d7	d13	d-8	d7	d13
ACTH	pg/ml	72	129	97	157	233	141
CORTISOL	nmol/l	1536	1220	1202	1222	1705	1128
ALDOST	pg/ml	185	948	523	155	1073	457
INSULIN	mU/l	12.0	8.0	9.0	20.0	18.0	24.0
GLUCAG	pg/ml	585	295	258	619	594	303
C-PEPTI	ng/ml	n/a	1.60	1.00	n/a	1.50	2.20
GASTRIN	pg/ml	n/a	83	84	n/a	91	84
T3	nmol/l	1.17	1.68	1.51	1.43	1.51	2.00
T4	nmol/l	58	76	60	61	87	60
TSH	mUI/l	0.81	1.31	1.16	0.08	0.34	0.41
IPH	pg/ml	59	47	58	145	82	53
CT	pg/ml	3.10	6.40	4.90	7.00	3.60	2.30
VD25-H	nmol/l	61	43	40	72	56	60
VD1-25dh	pmol/l	n/a	-	-	n/a	-	-
OSTEO	ng/ml	n/a	21	25	n/a	35	35
CTx	nmol/l	12	21	25	17	34	28
ICTP	ng/ml	28	28	24	29	30	24
PICP	ng/ml	n/a	115	142	n/a	240	287
G.H.	ng/ml	6.3	15.2	8.6	5.1	17.9	13.1
S.STA	pg/ml	n/a	-	-	n/a	-	-
S.MED	**ng/ml**	**n/a**	**374**	**297**	**n/a**	**204**	**488**
PROLACT	ng/ml	0.00	2.30	4.30	19.30	20.20	24.40
TESTO	nmol/l						
ESTR	nmol/l	47	63	59	141	82	170
PROG	pmol/l	1.80	1.90	1.50	2.60	4.00	1.60

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

TABLE 10
Hormones – Females

PTS893

Animal no.			W62555			W62556	
Test	Units	d-8	d7	d13	d-8	d7	d13
ACTH	pg/ml	109	104	110	95	132	126
CORTISOL	nmol/l	1482	1331	917	1532	1253	1375
ALDOST	pg/ml	314	217	330	210	228	226
INSULIN	mU/l	1.0	22.0	19.0	15.0	30.0	22.0
GLUCAG	pg/ml	711	591	657	696	437	380
C-PEPTI	ng/ml	n/a	3.00	2.40	n/a	3.80	3.50
GASTRIN	pg/ml	n/a	83	82	n/a	96	91
T3	nmol/l	2.08	2.74	2.63	1.98	2.69	2.05
T4	nmol/l	72	56	55	59	61	45
TSH	mUI/l	0.34	0.14	0.25	0.88	0.89	0.69
IPH	pg/ml	95	45	64	111	67	58
CT	pg/ml	2.50	1.90	2.70	1.80	2.90	2.80
VD25-H	nmol/l	72	53	47	55	44	43
VD1-25dh	pmol/l	n/a	-	-	n/a	-	-
OSTEO	ng/ml	n/a	38	43	n/a	32	36
CTx	nmol/l	13	11	15	17	14	14
ICTP	ng/ml	22	16	16	20	15	15
PICP	ng/ml	n/a	612	436	n/a	478	393
G.H.	ng/ml	3.5	1.5	0.0	1.1	8.2	11.8
S.STA	pg/ml	n/a	-	-	n/a	-	-
S.MED	**ng/ml**	n/a	**533**	**502**	n/a	**432**	**589**
PROLACT	ng/ml	0.00	0.20	3.20	9.90	5.70	3.60
TESTO	nmol/l						
ESTR	nmol/l	67	68	60	59	66	57
PROG	pmol/l	2.80	1.70	1.50	2.40	2.20	2.40

d-8, d7 and d13 indicate day -8, day 7 and day 13 relative to the starting day of dosing

[0053] *Tissue sampling.* Animals were killed by deep anaesthesia induced by intravenous injection of Pentothal®, followed by exsanguinations. All relevant tissues were sampled for histopathology and gene expression profiling. The following tissue samples were processed for analysis: liver, kidney, pituitary, muscle, bone, duodenum, spleen and trachea. Samples for histopathology were fixed in phosphate-buffered 10% formalin. Bone demineralization was performed with 10% formic acid. Tissue samples were embedded in Paraplast® and sectioned at 4 microns, for staining with haematoxylin and eosin. Samples for gene expression profiling were quickly frozen in liquid nitrogen immediately after excision, stored on dry ice and subsequently in a deep-freezer at approximately -80°C until further use. All selected tissues for gene expression profiling were examined histopathologically.

[0054] *Histopathology*. Histopathological examination of the tissues selected for gene profiling analysis exhibited a normal spectrum of incidental lesions which were in terms of severity and distribution of lesions not different to the controls in all groups of treatment.

[0055] A slightly higher incidence of inflammatory and regenerative changes in the kidneys of females administered salmon calcitonin was observed. These changes were not considered to be relevant, since no records of kidney toxicity exist after 40 years of calcitonin therapeutic use.

[0056] Bone sections were stained for osteonectin, osteopontin and osteocalcin and were evaluated histopathologically. Histomorphometry of the bone tissue was performed regarding parameters for bone resorption and synthesis (osteoid formation).

[0057] The osteonectin, osteopontin, and osteocalcin staining of the tibia showed no difference between the groups one (control) and two (salmon calcitonin). Osteonectin exhibited a major enlargement and deterioration of the epiphysial growth plate of animal no 2553 due to a severe non-treatment related pathological status (severe, subacute epiphysiolysis).

[0058] Histomorphometry of bone tissue was performed to determine parameters related to bone resorption and bone synthesis (osteoid formation).

[0059] The results (see, TABLES 11 and 12) showed that salmon calcitonin increased trabecular volume and thickness in about a 17% in tibia, but not in vertebra. PTS893 reduced the cortical thickness (18%) and increased the cortical porosity (54%) in tibia (T), but not in vertebra (V). In contrast, PTS893 induced an increase in osteoid volume (37%T, 213%V) and surface (49%T, 37%V), as well as an increase in the osteoblast surface (40%T, 24%V), in both tibia and vertebra, respectively.

TABLE 11
Histomorphometry Tibia (Average Males and Females)

	BT/TV %	Tb Th µm	Tb N mm^{-1}	Tb Sp µm	Ct Por %	Ct Th µm	OS/BS %	OV/B V %	ES/BS %	Obs/BS %
Control	20.70	106.32	1.95	407.20	2.53	1583.13	40.00	8.76	5.73	17.53
	17.72	97.99	1.81	454.90	2.59	976.66	33.37	8.51	4.70	12.77
	28.74	109.18	2.63	270.69	1.21	1036.24	29.45	5.79	10.19	11.70
	20.15	103.59	1.94	410.62	1.19	1031.89	29.19	5.29	5.71	15.80
mean	21.83	104.27	2.08	385.85	1.88	1156.98	33.00	7.09	6.58	14.45
SD	4.79	4.77	0.37	79.79	0.78	285.39	5.04	1.80	2.45	2.69
sCT	32.28	140.64	2.30	295.01	2.10	895.98	42.71	11.72	5.02	18.32
	25.00	122.19	2.05	366.51	1.98	1022.55	31.58	5.86	2.31	6.37
	29.96	129.05	2.32	301.75	1.61	939.32	35.21	5.03	6.89	18.58
	16.08	115.65	1.39	603.45	2.40	1178.70	30.37	4.01	5.61	19.36
mean	25.83	126.88	2.01	391.68	2.02	1009.14	34.97	6.65	4.95	15.66
SD	7.17	10.68	0.43	144.81	0.33	124.65	5.56	3.46	1.93	6.21
PTS893	19.69	129.22	1.52	526.99	2.76	1022.62	54.84	11.24	4.62	16.16
	16.65	93.20	1.79	466.69	2.94	893.43	43.57	9.61	4.76	21.25
	25.74	120.52	2.13	347.63	2.94	950.33	43.63	8.14	4.21	18.46
	24.78	126.07	1.97	382.61	2.95	939.53	54.97	9.95	2.85	25.25
mean	21.72	117.25	1.85	430.98	2.90	951.48	49.25	9.74	4.11	20.28
SD	4.30	16.43	0.26	81.20	0.09	53.46	6.53	1.28	0.87	3.91

sCT: salmon Calcitonin; SD: Standard deviation
BV/TV trabecular bone volume; Tb. Th. Trabecular thickness; Tb. N. Trabecular number; Tb. Sp. Trabecular Separation; Ct. Por. Cortical porosity; Ct, Th. Cortical thickness; OS/BS osteoid surface; OV/BV osteoid volume; ES/BS eroded surface; Obs/BS osteoblast surface.

TABLE 12
Histomorphometry Vertebra (Average Males and Females)

	BT/TV %	Tb Th µm	Tb N mm^{-1}	Tb Sp µm	Ct Por %	Ct Th µm	OS/BS %	OV/BV %	ES/BS %	Obs/BS %
Control	21.67	179.80	1.21	649.79	0.88	887.91	23.61	1.22	8.94	16.14
	15.85	144.89	1.09	769.35	0.26	639.93	20.77	2.02	8.81	5.96
	19.54	122.91	1.59	506.23	0.87	416.48	17.91	1.58	5.85	4.07
	21.95	131.30	1.67	466.91	0.85	604.45	11.58	0.97	1.82	4.79
mean	19.75	144.72	1.39	598.07	0.71	637.20	18.47	1.45	6.36	7.74
SD	2.82	25.07	0.28	138.62	0.30	193.78	5.15	0.45	3.34	5.65
sCT	17.32	113.29	1.53	540.84	1.70	705.10	3.95	0.46	11.60	3.21
	19.33	144.31	1.34	602.15	1.18	810.09	5.82	0.86	2.55	3.97
	20.11	118.49	1.70	470.71	1.18	576.42	11.48	1.43	4.93	6.81
	19.46	123.71	1.57	511.96	0.12	907.16	4.91	0.32	3.47	1.23
mean	19.06	124.95	1.53	531.42	1.05	749.69	6.54	0.77	5.64	3.80
SD	1.21	13.59	0.15	55.24	0.66	141.96	3.38	0.50	4.09	2.31
PTS893	15.15	105.46	1.44	590.67	1.49	707.43	18.84	3.24	9.31	10.36
	20.23	118.79	1.70	468.39	1.45	629.35	41.28	8.42	2.30	9.07
	23.56	134.66	1.75	436.79	0.41	740.87	23.65	3.49	2.55	10.47
	24.86	134.82	1.84	407.56	0.92	624.35	17.66	2.66	3.96	8.33
mean	20.95	123.43	1.68	475.85	1.07	675.50	25.36	4.45	4.53	9.56
SD	4.33	14.15	0.17	80.47	0.51	57.85	10.93	2.67	3.27	1.04

sCT: salmon Calcitonin; SD: Standard deviation
BV/TV trabecular bone volume; Tb. Th. Trabecular thickness; Tb. N. Trabecular number; Tb. Sp. Trabecular Separation; Ct. Por. Cortical porosity; Ct, Th. Cortical thickness; OS/BS osteoid surface; OV/BV osteoid volume; ES/BS eroded surface; Obs/BS osteoblast surface.

[0060] Histomorphometry showed inconsistent results between tibial and vertebral bone, except for an increase in osteoid synthesis induced by PTS893. This effect is well documented for parathyroid hormone, when administered in a discontinuous way.

[0061] *RNA extraction and purification.* A set of tissues was selected for gene expression profiling. These set included samples from kidney, bone, muscle, duodenum, pituitary and liver. In particular, diaphyseal bone from femur and tibia were processed for gene expression profiling. Briefly, total RNA was obtained by acid guanidinium thiocyanate-phenol-chloroform extraction (Trizol®, Invitrogen Life Technologies, Carlsbad, Calif. USA) from each frozen tissue section and the total RNA was then purified on an affinity resin (RNeasy®, Qiagen) according to the manufacturer's instructions. Total RNA was quantified by the absorbance at $\lambda = 260$ nm (A260nm), and the purity was estimated by the ratio A260nm/A280nm. Integrity of the RNA molecules was confirmed by non-denaturing agarose gel electrophoresis. RNA was stored at approximately -80°C until analysis. One part of each individual RNA sample was kept for the analysis of critical genes by means of Real-time PCR.

[0062] *Hybridization assay.* Transcript profiling by means of GeneChip® expression probe arrays was done in the laboratories of the Genomics Factory EU, as recommended by the manufacturer of the GeneChip® system (*GeneChip Expression Analysis Technical Manual*, Affymetrix Inc., Santa Clara, Calif. USA). HG-U95Av2 GeneChip® expression probe arrays (Affymetrix, Santa Clara Calif. USA) were used. Double stranded cDNA was synthesized with a starting amount of approximately 5 µg full-length total RNA using the Superscript Choice System (Invitrogen Life Technologies) in the presence of a T7-(dT) 24 DNA oligonucleotide primer. Following synthesis, the cDNA was purified by phenol/chloroform/isoamylalcohol extraction and ethanol precipitation. The purified cDNA was then transcribed in vitro using the BioArray® High Yield RNA Transcript Labelling Kit (ENZO) in the presence of biotinylated ribonucleotides form biotin labelled cRNA. The labelled cRNA was then purified on an affinity resin (Rneasy®, Qiagen), quantified and fragmented. An amount of approximately 10 µg labelled cRNA was hybridized for approximately 16 hours at 45°C to an expression probe array. The array was then washed and stained twice with streptavidin-phycoerythrin (Molecular Probes) using the GeneChip Fluidics

Workstation 400 (Affymetrix). The array was then scanned twice using a confocal laser scanner (GeneArray® Scanner, Agilent) resulting in one scanned image. This resulting ".data-file" was processed using the Micro Array Analysis Suite version 4 (MAS4) program (Affymetrix) into a ".cel-file". The ".cel file" was captured and loaded into the Affymetrix GeneChip Laboratory Information Management System (LIMS). The LIMS database is connected to a UNIX Sun Solaris server through a network filing system that allows for the average intensities for all probes cells (CEL file) to be downloaded into an Oracle database. Raw data was converted to expression levels using a "target intensity" of 150. The numerical values displayed are weighted averages of the signal intensities of the probe-pairs comprised in a probe-set for a given transcript sequence (AvgDiff value). The data were checked for quality and loaded into the GeneSpring® software versions 4.2.4 and 5 (Silicon Genetics, Calif. USA) for analysis.

[0063] *Data analysis*. Data analysis was performed with the Silicon Genetics software package GeneSpring version 4.2.1 and 5. Average difference values below 20 were set to 20. Various filtering and clustering tools in these programs were used to explore the data sets and identify transcript level changes that inform on altered cellular and tissue functions and that can be used to establish working hypotheses on the modes of action of the compound.

[0064] The threshold range for considering as up or down regulation was determined within the context of the biological interpretation of the EXAMPLE.

[0065] The information content of these data sets is a conjunction of numerical changes and biological information. The decision to consider a specific gene relevant was based on a conjunction of numerical changes identified by comparative and statistical algorithms and the relationship to other modulated genes that point to a common biological theme. The weight of that relationship was assessed by the analyst through a review of the relevant scientific literature.

[0066] Increase and decrease reported here refer to transcript abundance, unless specifically stated.

[0067] *Gene expression profiling.* Multi-organ comparative gene profiling analysis was performed in the group administered salmon calcitonin at 50 µg/animal/day. The organs chosen for analysis were liver, kidney, pituitary, skeletal muscle, bone, duodenum, spleen and trachea.

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
36611_at	acid phosphatase 1 isoform a			-1.33		-1.33	
32714_s_at	activin A receptor type II-like 1	-1.62			-1.83		
39314_at	activin A type IIB receptor precursor			-1.12	1.41	-4.15	
35915_at	activin beta-C chain.	-1.21			-2.41	-1.67	
36621_at	alpha-2-HS-glycoprotein	1.33	1.53				1.12
34588_i_at	amelogenin			-1.61			
37747_at	annexin V		-1.30	1.87		-2.58	
40376_at	arylsulfatase E precursor		-1.59				
39326_at	ATPase H(+)- vacuolar	-1.57	-2.80			-1.62	
38814_at	ATPase H(+)- vacuolar subunit	1.22					
33741_at	ATPase, H+ transport, lysosomal	1.23				-1.50	
33033_at	ATPase, H+ transporting, lysosomal	-1.29		-3.19		-1.43	1.23
38814_at	ATPase, H+ transporting, lysosomal				1.30	-1.28	1.14
38126_at	biglycan					1.75	-1.61
39407_at	bone morphogenetic protein 1			-1.20		-1.55	
31399_at	bone morphogenetic protein 10	1.44	1.45	-1.31	-1.77		
1113_at	bone morphogenetic protein 2A	-1.12	2.63				1.29
1831_at	bone morphogenetic protein 5	-1.43	1.39	1.40			
1733_at	bone morphogenetic protein 6 precursor		-1.37	-1.17	-1.64	-1.27	-1.1
34500_at	calcium binding protein 1 (calbrain)		2.31			1.21	
31670_s_at	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma		1.17		1.57	-1.28	1.60
1751_g_at	calreticulin	-4.03	-1.60		1.67		
32067_at	cAMP responsive element modulator (CREM)	1.39		-1.24		-1.50	
39241_at	carbonic anhydrase I	-2.68		1.18		-1.69	
40095_at	carbonic anhydrase II	-1.69					
40163_r_at	cartilage oligomeric matrix protein precursor	2.36	5.61				
128_at	cathepsin k	1.18			1.35	-2.33	
129_g_at	cathepsin k	1.20	-1.54		1.17	-1.28	
38466_at	cathepsin k	1.27			1.40		-1.19
40718_at	cathepsin w	-1.31		-1.54		2.05	
32833_at	CDC-like kinase 1	1.63					
646_s_at	CDC-like kinase 2 isoform hclk2/139	1.19			1.86		
38112_g_at	chondroitin sulphate proteoglycan 2 (versican)		-2.16		1.51		-1.68
32642_at	chondroitin sulphate proteoglycan 3 (neurocan)					-1.49	

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
31493_s_at	chorionic somatomammotropin hormone 1					-1.59	
40714_at	chymotrypsin C (caldecrin)					1.39	3.22
35474_s_at	Collagen type 1 and PDGFB fusion transcript				-7.30		-3.35
598_at	collagen type II alpha-1	-1.38		1.69	-1.27	2.77	-3.02
32488_at	collagen type III alpha 1	-1.41	-1.59	-1.53	-3.20	-1.89	-1.35
38952_s_at	collagen type IV alpha-2	1.23				-1.73	
35379_at	collagen type IX alpha1	-2.22	-3.28				
38722_at	collagen type VI alpha-1		-3.38	-1.13	-1.42		
34802_at	collagen type VI alpha-2 (AA 570-998)	-1.37		-1.10	-1.39		-1.28
37892_at	collagen type XI alpha-1	1.24				-2.46	-1.51
1026_s_at	collagen type XI alpha2	-1.20	-1.32			1.15	-2.20
1027_at	collagen type XI alpha2	1.11		-1.25			1.37
32305_at	collagen, type I, alpha 2		-1.45				-1.54
39333_at	collagen, type IV, alpha 1						-1.49
39925_at	collagen, type IX, alpha 2					-2.38	-1.36
38420_at	collagen, type V, alpha 2		-1.29	-1.18	-1.11		-1.10
41351_at	collagen, type VI, alpha 1		-2.29		-1.27	-1.50	
41350_at	collagen, type VI, alpha 1 precursor						-3.55
35168_f_at	collagen, type XVI, alpha 1						-1.59
35169_at	collagen, type XVI, alpha 1						-1.18
39632_at	collagenase 3 (matrix metalloproteinase 13)	1.20					
36638_at	connective tissue growth factor					-2.11	
40697_at	cyclin A2	-1.60					
34736_at	cyclin B1	-2.83					
36650_at	cyclin D2	1.21					
35249_at	cyclin E2	-2.95					
1206_at	cyclin-dependent kinase 5	1.56					-1.54
799_at	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	1.32					
41546_at	cyclin-dependent kinase 6	1.15	1.52			1.34	
2031_s_at	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.95					
35816_at	cystatin B (stefin B)	1.57					
806_at	cytokine-inducible kinase	1.20				1.35	
40049_at	death-associated protein kinase 1					-1.47	-1.29
33903_at	death-associated protein kinase 3	-1.22					
34029_at	dentin matrix acidic phosphoprotein 1 (DMP1)	1.65					
40186_at	dual specificity phosphatase 9						1.59
37996_s_at	dystrophia myotonica-protein kinase		1.25				-1.50
342_at	ectonucleotide Pyrophosphatase/ Phosphodiesterase 1;	1.45					

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
343_s_at	ectonucleotide pyrophosphatase/ phosphodiesterase 1;	1.11			-1.42		
33602_at	endothelial differentiation, G protein coupled receptor 6 precursor		1.15			2.24	-1.66
1442_at	oestrogen receptor	1.47	1.23			1.60	
33670_at	oestrogen receptor	1.30					
1487_at	oestrogen receptor-related protein	1.11			-1.52		1.24
38882_r_at	oestrogen-responsive B box protein (EBBP)	1.22		-1.51			
39945_at	fibroblast activation protein	-1.27				-1.48	-1.32
996_at	fibroblast growth factor 1 (acidic)		1.17		-1.41		
41586_at	fibroblast growth factor 18		2.06				
1730_s_at	fibroblast growth factor 4		1.55				1.46
424_s_at	fibroblast growth factor receptor.	-1.17	-1.59				
40131_at	follistatin-like 1	-1.31					
40132_g_at	follistatin-like 1					-1.22	1.15
33510_s_at	glutamate receptor, metabotropic 1	1.26			-1.31		
33269_at	GPI1 N-acetylglucosaminyl transferase component Gpi1	1.24					
1401_g_at	granulocyte-macrophage colony-stimulating factor (CSF1)	-3.07	2.24				
1911_s_at	growth arrest and DNA-damage-inducible, alpha		1.84		-3.84		1.24
37615_at	growth factor receptor-bound protein 10	1.21				-1.61	
32845_at	heparan sulphate proteoglycan 2 (perlecan)			1.27			-1.11
32778_at	inositol 1,4,5-triphosphate receptor, type 1		1.75			-2.57	1.20
32779_s_at	inositol 1,4,5-triphosphate receptor, type 1			1.21	2.02		
756_at	inositol 1,4,5-triphosphate receptor, type 2						1.24
34209_at	inositol 1,4,5-trisphosphate 3-kinase isoenzyme		2.29		1.42	-1.36	1.75
33506_at	inositol polyphosphate 4-phosphatase type I-beta	1.12	1.66	2.09		1.27	
172_at	inositol polyphosphate-5-phosphatase,			-1.22	-1.15		
32697_at	inositol(myo)-1(or 4)-monophosphatase 1	-1.36	-2.70			1.61	
36496_at	inositol(myo)-1(or 4)-monophosphatase 2						1.13
2079_s_at	insulin-like growth factor (IGF-II)				-1.32	1.15	-1.31
36782_s_at	insulin-like growth factor 2 (somatomedin A)						-1.69
1232_s_at	insulin-like growth factor binding protein	-1.31			-1.53		

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
40422_at	insulin-like growth factor binding protein 2					-2.97	-1.16
1586_at	insulin-like growth factor binding protein 3	1.45		-1.16	1.70		
37319_at	insulin-like growth factor binding protein 3	2.17			1.58		-1.52
41420_at	insulin-like growth factor binding protein 5		1.15		-2.66		
1741_s_at	insulin-like growth factor binding protein-2	-2.49				-2.17	-1.22
1464_at	insulin-like growth factor II precursor	1.18	1.10			-1.26	
1591_s_at	insulin-like growth factor II precursor	1.41					-2.80
33082_at	integrin alpha 10 subunit	1.33			-2.32	-1.18	
1100_at	interleukin-1 receptor-associated kinase				1.39	-1.48	
2005_s_at	Janus kinase 3				-1.51		1.57
40060_r_at	LIM protein (similar to rat protein kinase C-binding enigma)	1.44				-1.68	-1.31
36811_at	lysyl oxidase-like protein	-1.44	1.14		1.30	-1.19	
1433_g_at	MAD, mothers against decapentaplegic homolog 3	1.14	-1.13		-1.61	-1.65	-1.69
34655_at	MAGUKs (membrane-associated guanylate kinase homologues	1.23					
35652_g_at	MAP kinase kinase kinase (MTK1)	1.14					
33246_at	MAPK13: mitogen-activated protein kinase 13			-1.24	-1.13	-1.91	1.65
41280_r_at	MAPK8IP1: mitogen-activated protein kinase 8 interacting protein 1	-1.31	1.92			1.58	
2004_at	MEK kinase				1.13	-1.62	1.16
1509_at	metalloproteinase	-1.42		-1.11		-1.23	-1.18
976_s_at	mitogen-activated protein kinase 1	-1.61					
34006_s_at	mitogen-activated protein kinase 8	1.32					
1844_s_at	mitogen-activated protein kinase kinase 1					-1.60	1.15
35694_at	mitogen-activated protein kinase kinase kinase kinase 4				1.26		
1469_at	mitogen-activated protein kinase-activated protein kinase 2		1.13	-1.30			1.16
1637_at	mitogen-activated protein kinase-activated protein kinase 3				1.11		1.34
37565_at	MMD: monocyte to macrophage differentiation-associated	1.28	-2.48				-1.28
38307_at	neurochondrin,			2.80		-1.39	
39144_at	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1		2.72		1.42		-1.70
41202_s_at	OS-4 protein (OS-4)	1.24				-1.72	
1451_s_at	OSF-2os osteoblast specific factor-2 (periostin)	-1.65				-2.06	1.56

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
467_at	osteoclast stimulating factor (OSF)	-1.23	-1.50	-1.58		-4.12	
33814_at	PAK4	1.16			-1.33	1.11	
38757_at	PDGF associated protein.	-1.89				-1.15	1.20
146_at	phosphatidylinositol 4-kinase, catalytic, beta polypeptide				1.19		1.23
34496_at	phosphatidylinositol glycan, class L		2.34	1.34	1.51		
34169_s_at	phosphatidylinositol polyphosphate 5-phosphotase, isoform b					-1.33	1.49
37412_at	phosphatidylinositol-4-phosphate 5-kinase isoform C (-1)	-1.87		-1.31			
37253_at	phosphatidylinositol-4-phosphate 5-kinase, type I, beta		1.17		-1.13		1.11
35741_at	phosphatidylinositol-4-phosphate 5-kinase, type II, beta				-1.18		-1.18
751_at	phosphatidylinositol-glycan-class C (PIG-C)	1.14	1.19			-1.22	
666_at	phosphodiesterase 4A, cAMP-specific	1.33		-1.32		-1.18	
38526_at	phosphodiesterase 4D, cAMP-specific (dunce (Drosophila)-homolog phosphodiesterase E3)	1.30	1.15				3.51
38921_at	phosphodiesterase IB, calmodulin-dependent	1.52				1.42	1.12
31699_at	phosphoinositide-3-kinase	1.56		-1.56			
36287_at	phosphoinositide-3-kinase, catalytic, gamma polypeptide	1.31					
35665_at	phosphoinositide-3-kinase, class 3					-1.11	1.21
364_s_at	phospholipase C b3	1.22					
901_g_at	phospholipase C, beta 4	-1.20		1.41		-1.55	
1293_s_at	phospholipase D	-1.26					
38023_at	phosphotidylinositol transfer protein		2.25		1.33	1.55	1.71
38269_at	PKD2 Protein kinase D2			1.34			
32306_g_at	preprocollagen type I alpha-2	1.19		-1.38		-1.75	-1.31
35473_at	preprocollagen type I alpha1.	-2.72	-1.37		-3.94		-2.70
32307_s_at	procollagen	1.13		-1.26	-2.44	-1.56	-1.82
37605_at	procollagen alpha 1 type II				-1.84		-1.61
36184_at	procollagen-lysine 5-dioxygenase		2.52		-2.15		-1.30
37037_at	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide I			1.87	1.46	-1.67	1.29
37633_s_at	progestagen-associated endometrial protein (placental protein 14, pregnancy-associated endometrial alpha-2-globulin, alpha uterine protein)		2.00				
36109_at	prolidase (imidodipeptidase) PEPD:	-2.55				-2.05	
1884_s_at	proliferating cell nuclear antigen	-1.85					
36666_at	prolyl 4-hydroxylase beta	1.95			1.37	2.08	

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
718_at	protease, serine, 11 (IGF binding)	-1.30				-1.81	-1.30
719_g_at	protease, serine, 11 (IGF binding)	-1.43				-1.97	-1.27
385_at	proteasome (prosome, macropain) subunit, beta type, 10	1.36		-1.29			
37431_at	protein inhibitor of activated STAT X					-1.23	1.28
39183_at	protein kinase 1 PCTAIRE	-1.17					
39711_at	protein kinase C substrate 80K-H						1.31
1437_at	protein kinase C, alpha			-2.06			1.82
36359_at	protein kinase, cAMP-dependent, catalytic, gamma	1.39		1.14	-1.49	1.30	1.13
1091_at	protein kinase, cAMP-dependent, regulatory, type I, beta	1.65	-1.80		2.06		
116_at	protein kinase, cAMP-dependent, regulatory, type II, alpha	1.28			-1.18		
33633_at	purinergic receptor P2Y, G-protein coupled, 11	1.90	-1.82				
32737_at	RAC2 Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	1.16					1.22
1007_s_at	receptor tyrosine kinase DDR	1.21					
1048_at	retinoid X receptor-gamma	1.47				1.47	
41404_at	ribosomal protein S6 kinase	-1.67	-1.40		-1.83		-1.40
865_at	ribosomal protein S6 kinase, 90kD, polypeptide 3		-1.42				1.27
32290_at	SCAMP1: secretory carrier membrane protein 1 (vesicular transport)	2.50				-1.27	-1.39
34342_s_at	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)		1.15				-3.01
39166_s_at	serine (or cysteine) proteinase inhibitor, clade H (heat shock protein 47), member 2		-2.82	1.56	2.04		-1.29
36217_at	serine/threonine kinase 38	1.54					-1.59
1223_at	serine/threonine protein kinase			2.42			
32447_at	SF-1; Steroidogenic factor-1	8.76		1.59		1.27	-2.01
33338_at	signal transducer and activator of transcription 1			-1.14	1.15	-2.11	-1.93
1244_at	signal transducer and activator of transcription 2, 113kD						1.57
40458_at	signal transducer and activator of transcription 5A					1.14	1.39
506_s_at	signal transducer and activator of transcription 5A				1.32	2.60	
41222_at	signal transducer and activator of transcription 6 (STAT6)	1.44			1.14	-1.46	
1950_s_at	Smad 3				-2.44		-1.16

TABLE 13
Multi-Organ Gene Expression Profiling of Salmon Calcitonin

GeneChip® expression probe set identifier	Coding Gene	bone	kidney	liver	muscle	pituitary	trachea
38889_at	Smad anchor for receptor activation, isoform 1		1.28			-1.14	-1.51
1013_at	Smad5					-2.62	1.22
1955_s_at	SMAD6 (inhibits BMP/Smad1 (MADH1)	1.19				-1.37	
37718_at	SNF-1 related kinase	1.49		-1.13	1.18		
35883_at	Spi-B transcription factor (SPI1/PU.1 related)		3.76			-2.96	1.15
472_at	Stat5b (stat5b)	-1.42	-1.28			-1.83	-2.50
38669_at	Ste20-related serine/threonine kinase	1.24				-1.78	
38374_at	TEIG; TGFB inducible early growth response	1.18				-1.79	
224_at	TGFB inducible early growth response; TIEG	1.26				-2.69	
36940_at	TGFB1-induced anti-apoptotic factor 1	1.22			1.28	-1.38	
32217_at	TGF-beta induced apoptosis protein 12	1.40	1.55		1.12		
41445_at	TGF-beta precursor	1.14	1.11				
1890_at	TGF-beta superfamily protein	1.74	1.85	1.12		1.38	
40631_at	Tob	-1.14			1.28	-2.09	
32219_at	tousled-like kinase 1		-1.16				
1897_at	transforming growth factor, beta receptor III (betaglycan, 300kD)		1.18				1.12
1735_g_at	transforming growth factor-beta 3		-1.15		-4.45	-1.39	-2.23
1767_s_at	transforming growth factor-beta 3 (TGF-beta 3)	-1.71	1.41		-1.71		
40581_at	TRIO: triple functional domain (PTPRF interacting)	1.65	1.62		1.34		-1.42
32272_at	tubulin alpha	-1.20			1.18		
330_s_at	tubulin alpha 1	-1.80		1.23	-1.20	-1.19	
40567_at	tubulin alpha 3	-1.39		-1.18			-1.10
685_f_at	tubulin alpha isotype H2-alpha	-4.36		1.32		2.13	
151_s_at	tubulin beta	-1.40	-1.14	1.16	1.22	1.16	
33678_i_at	tubulin beta 2	-1.15			1.75		
33679_f_at	tubulin beta 2	-1.31			1.45		
709_at	tubulin beta 3	-1.18				-1.35	1.20
471_f_at	tubulin beta 4	-1.38			1.50		
39399_at	tubulin beta, cofactor D	-1.85				-4.69	
32098_at	type VI collagen alpha 2 chain precursor						-3.79
1651_at	ubiquitin carrier protein E2-C	-3.74					
1953_at	vascular endothelial growth factor	1.40					
36101_s_at	vascular endothelial growth factor	1.45					
37268_at	vascular endothelial growth factor B				-1.58		
36140_at	Y box binding protein-1	2.30	1.86		2.36	-2.72	

[0068] In addition, the effect of PTS893 was assessed in bone.

TABLE 14
Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

GeneChip® Expression Probe Set Identifier	Coding Gene	Fold Increase Salmon Calcitonin	Fold Increase PTS893
38909_at	25-hydroxyvitamin D3 1-alpha-hydroxylase		-1.14
32714_s_at	activin A receptor type II-like 1	-1.62	
35915_at	activin beta-C chain.	-1.21	
39279_at	activin type II receptor		1.24
39383_at	adenylate cyclase 6, isoform a		-1.22
38965_at	aggrecan 1		2.03
39206_s_at	aggrecan 1		1.41
36621_at	alpha-2-HS-glycoprotein	1.33	
34589_f_at	Amelogenin	1.10	-3.10
39326_at	ATPase H(+) vacuolar	-1.57	-1.19
38814_at	ATPase H(+) vacuolar	1.22	
33741_at	ATPase, H+ transport, lysosomal	1.23	
33033_at	ATPase, H+ transporting, lysosomal	-1.29	-1.17
40328_at	bHLH transcription factor		2.57
39407_at	bone morphogenetic protein 1		1.16
31399_at	bone morphogenetic protein 10	1.44	1.20
1113_at	bone morphogenetic protein 2A	-1.12	-1.13
40367_at	bone morphogenetic protein 2A		-1.18
1114_at	bone morphogenetic protein 2B or BMP4		-1.70
1831_at	bone morphogenetic protein 5	-1.43	-1.60
1733_at	bone morphogenetic protein 6 precursor		1.27
40333_at	bone morphogenetic protein-4 (hBMP-4)		-1.42
34847_s_at	calcium/calmodulin-dependent protein kinase (CaM kinase) II beta		1.13
33935_at	calcyclin binding protein		1.41
1751_g_at	Calreticulin	-4.03	
32067_at	cAMP responsive element modulator (CREM)	1.39	2.75
39241_at	carbonic anhydrase I	-2.68	
40095_at	carbonic anhydrase II	-1.69	
40163_r_at	cartilage oligomeric matrix protein precursor	2.36	
128_at	cathepsin k	1.18	
129_g_at	cathepsin k	1.20	
38466_at	cathepsin k	1.27	
40718_at	cathepsin w	-1.31	
32833_at	CDC-like kinase 1	1.63	
646_s_at	CDC-like kinase 2 isoform hclk2/139	1.19	
34763_at	chondroitin sulphate proteoglycan 6		-1.18
598_at	collagen type II alpha-1	-1.38	-1.19
32488_at	collagen type III alpha 1	-1.41	
38952_s_at	collagen type IV alpha-2	1.23	1.44
35379_at	collagen type IX alpha1	-2.22	
34802_at	collagen type VI alpha-2 (AA 570-998)	-1.37	
38566_at	collagen type X alpha-1		1.67
37892_at	collagen type XI alpha-1	1.24	1.18
1026_s_at	collagen type XI alpha2	-1.20	

TABLE 14
Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

GeneChip® Expression Probe Set Identifier	Coding Gene	Fold Increase Salmon Calcitonin	Fold Increase PTS893
1027_at	collagen type XI alpha2	1.11	
39632_at	collagenase 3 (matrix metalloproteinase 13)	1.20	
36638_at	connective tissue growth factor.		-1.32
1943_at	cyclin A		-1.74
40697_at	cyclin A2	-1.60	-1.39
34736_at	cyclin B1	-2.83	
39251_at	cyclin C		-2.03
1983_at	cyclin D2		-1.28
36650_at	cyclin D2	1.21	
35249_at	cyclin E2	-2.95	
1649_at	cyclin G1 interacting protein		1.31
1913_at	cyclin G2		-1.29
160024_at	cyclin-dependent kinase (CDC2-like) 10 PISSLRE		1.53
1942_s_at	cyclin-dependent kinase 4		-1.22
1206_at	cyclin-dependent kinase 5	1.56	
40549_at	cyclin-dependent kinase 5		-1.40
799_at	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	1.32	
41546_at	cyclin-dependent kinase 6	1.15	
2031_s_at	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.95	
1787_at	cyclin-dependent kinase inhibitor 1C		1.18
38673_s_at	cyclin-dependent kinase inhibitor 1C		1.13
39545_at	cyclin-dependent kinase inhibitor 1C		1.24
1797_at	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)		-1.21
35816_at	cystatin B (stefin B)	1.57	
806_at	cytokine-inducible kinase	1.20	
40049_at	death-associated protein kinase 1		-1.30
33903_at	death-associated protein kinase 3	-1.22	-7.73
34029_at	dentin matrix acidic phosphoprotein 1 (DMP1)	1.65	
38059_g_at	dermatopontin		1.72
343_s_at	ectonucleotide pyrophosphatase/ phosphodiesterase 1	1.11	
342_at	ectonucleotide Pyrophosphatase/ Phosphodiesterase 1	1.45	
1442_at	oestrogen receptor	1.47	
33670_at	oestrogen receptor	1.30	
1487_at	oestrogen receptor-related protein	1.11	
38882_r_at	oestrogen-responsive B box protein (EBBP)	1.22	
38902_r_at	oestrogen-responsive B box protein (EBBP)		1.23
39945_at	fibroblast activation protein	-1.27	
424_s_at	fibroblast growth factor receptor.	-1.17	
466_at	general transcription factor II,		1.34
1102_s_at	glucocorticoid receptor alpha		1.43
33510_s_at	glutamate receptor, metabotropic 1	1.26	1.23
33269_at	GPI1 N-acetylglucosaminyl transferase component Gpi1	1.24	1.21
41476_at	G-protein alpha subunit 11		1.24
1401_g_at	granulocyte-macrophage colony-stimulating factor (CSF1)	-3.07	-2.57
1911_s_at	growth arrest and DNA-damage-inducible protein (gadd45)		2.87
888_s_at	growth differentiation factor 1		-1.43
37615_at	growth factor receptor-bound protein 10	1.21	

TABLE 14
Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

GeneChip® Expression Probe Set Identifier	Coding Gene	Fold Increase Salmon Calcitonin	Fold Increase PTS893
33929_at	heparan sulphate proteoglycan (glypican).		2.00
39757_at	heparan sulphate proteoglycan core protein		1.10
755_at	inositol 1,4,5-trisphosphate receptor type 1		1.27
33506_at	inositol polyphosphate 4-phosphatase type I-beta	1.12	-1.24
33290_at	inositol polyphosphate 5-phosphatase (5ptase)		-1.20
32697_at	inositol(myo)-1(or 4)-monophosphatase 1	-1.36	
1975_s_at	insulin-like growth factor 1		-1.41
1501_at	insulin-like growth factor 1 (somatomedin C)		-1.12
1232_s_at	insulin-like growth factor binding protein	-1.31	
40422_at	insulin-like growth factor binding protein 2		-1.27
1586_at	insulin-like growth factor binding protein 3	1.45	
37319_at	insulin-like growth factor binding protein 3	2.17	
1737_s_at	insulin-like growth factor binding protein 4		1.13
41420_at	insulin-like growth factor binding protein 5		1.18
1396_at	insulin-like growth factor binding protein 5		1.62
1678_g_at	insulin-like growth factor binding protein 5		1.44
38650_at	insulin-like growth factor binding protein 5		1.53
1741_s_at	insulin-like growth factor binding protein-2	-2.49	-2.11
1464_at	insulin-like growth factor II precursor	1.18	
1591_s_at	insulin-like growth factor II precursor	1.41	1.31
39781_at	insulin-like growth factor-binding protein 4		1.16
33082_at	integrin alpha 10 subunit	1.33	
35131_at	integrin-binding sialoprotein (bone sialoprotein, bone sialoprotein II)		1.15
40060_r_at	LIM protein (similar to rat protein kinase C-binding enigma)	1.44	1.32
36184_at	lysyl hydroxylase (PLOD) procollagen-lysine, 2-oxoglutarate 5 dioxygenase		-1.40
34795_at	lysyl hydroxylase isoform 2 (PLOD2)		1.49
36811_at	lysyl oxidase-like protein	-1.44	
1433_g_at	MAD, mothers against decapentaplegic homolog 3	1.14	1.73
34655_at	MAGUKs (membrane-associated guanylate kinase homologues	1.23	
36179_at	MAP kinase activated protein kinase 2		1.18
35652_g_at	MAP kinase kinase kinase (MTK1)	1.14	
41279_f_at	MAPK8IP1 Mitogen-activated protein kinase 8 interacting protein 1		1.25
41280_r_at	MAPK8IP1: mitogen-activated protein kinase 8 interacting protein 1	-1.31	-1.31
1509_at	Metalloproteinase	-1.42	
976_s_at	mitogen-activated protein kinase 1	-1.61	1.12
34006_s_at	mitogen-activated protein kinase 8	1.32	
1439_s_at	mitogen-activated protein kinase-activated protein kinase 2		1.78
37565_at	MMD: monocyte to macrophage differentiation-associated	1.28	1.30
38369_at	myeloid differentiation primary response gene (88)		-1.10
1052_s_at	NF-IL6-beta protein		1.30
36472_at	N-myc and STAT interacter-		-1.35
38354_at	nuclear factor NF-IL6 (AA 1-345)		1.92

TABLE 14
Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

GeneChip® Expression Probe Set Identifier	Coding Gene	Fold Increase Salmon Calcitonin	Fold Increase PTS893
33106_at	nuclear orphan receptor LXR-alpha nuclear receptor subfamily 1, group H, member 3		3.29
33381_at	nuclear receptor co-activator		1.11
279_at	nuclear receptor subfamily 4, group A, member 1		2.30
280_g_at	nuclear receptor subfamily 4, group A, member 1		3.08
37623_at	nuclear receptor subfamily 4, group A, member 2 Member of the steroid/thyroid hormone receptor family		27.72
547_s_at	nuclear receptor subfamily 4, group A, member 2 Member of the steroid/thyroid hormone receptor family		26.77
190_at	nuclear receptor subfamily 4, group A, member 3 Member of steroid/thyroid receptor family of nuclear hormone receptors		5.45
41202_s_at	OS-4 protein (OS-4)	1.24	
1451_s_at	OSF-2os osteoblast specific factor-2 (periostin)	-1.65	
38822_at	O-sialoglycoprotein endopeptidase		2.43
467_at	osteoclast stimulating factor (OSF	-1.23	
35107_at	osteoprotegerin ligand		3.33
33814_at	PAK4 protein	1.16	
38757_at	PDGF associated protein.	-1.89	
40253_at	phosphatidylinositol 4-kinase (NPIK-C).	1.77	
37412_at	phosphatidylinositol-4-phosphate 5-kinase isoform C (-1)	-1.87	
751_at	phosphatidylinositol-glycan-class C (PIG-C)	1.14	-1.25
666_at	phosphodiesterase 4A, cAMP-specific	1.33	1.30
38526_at	phosphodiesterase 4D, cAMP-specific	1.30	3.53
38921_at	phosphodiesterase IB, calmodulin-dependent	1.52	
38944_at	phosphodiesterase IB, calmodulin-dependent		1.17
32029_at	phosphoinositide dependent protein kinase-1 (3)		1.16
31699_at	phosphoinositide-3-kinase	1.56	1.16
1085_s_at	phospholipase C		-1.14
364_s_at	phospholipase C b3	1.22	
901_g_at	phospholipase C, beta 4	-1.20	
1293_s_at	phospholipase D	-1.26	
32306_g_at	preprocollagen type I alpha-2	1.19	
35473_at	preprocollagen type I alpha1.	-2.72	
38951_at	PRKCQ Protein kinase C, theta		1.43
32307_s_at	procollagen	1.13	
34494_at	procollagen I-N proteinase.		1.92
37605_at	procollagen type II alpha1		1.91
36109_at	prolidase (imidodipeptidase) PEPD	-2.55	
1884_s_at	proliferating cell nuclear antigen	-1.85	
34390_at	prolyl 4-hydroxylase alpha (II) subunit		1.19
37037_at	prolyl 4-hydroxylase alpha subunit		1.20
36666_at	prolyl 4-hydroxylase beta	1.95	
36533_at	prostacyclin synthase		1.20
718_at	protease, serine, 11 (IGF binding)	-1.30	
719_g_at	protease, serine, 11 (IGF binding)	-1.43	
385_at	proteasome (prosome, macropain) subunit, beta type, 10	1.36	
39183_at	protein kinase 1 PCTAIRE	-1.17	

TABLE 14
Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

GeneChip® Expression Probe Set Identifier	Coding Gene	Fold Increase Salmon Calcitonin	Fold Increase PTS893
37698_at	protein kinase A (PRKA) anchor protein 1		1.29
39711_at	protein kinase C substrate 80K-H		1.13
39161_at	protein kinase Njmu-R1		1.21
35348_at	protein kinase, AMP-activated, beta 1 non-catalytic subunit		2.10
36359_at	protein kinase, cAMP-dependent, catalytic, gamma	1.39	
546_at	protein kinase, cAMP-dependent, catalytic, inhibitor alpha		1.14
227_g_at	protein kinase, cAMP-dependent, regulatory, type I, alpha		1.18
41768_at	protein kinase, cAMP-dependent, regulatory, type I, alpha		1.15
1091_at	protein kinase, cAMP-dependent, regulatory, type I, beta	1.65	
116_at	protein kinase, cAMP-dependent, regulatory, type II, alpha	1.28	
33633_at	purinergic receptor P2Y, G-protein coupled, 11	1.90	
32737_at	RAC2 Ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	1.16	
40299_at	RE2 G-protein coupled receptor		1.24
35668_at	receptor (calcitonin) activity modifying protein 1 RAMP 1		1.34
40696_at	receptor (TNFRSF)-interacting serine-threonine kinase 1		1.12
1007_s_at	receptor tyrosine kinase DDR	1.21	
37701_at	regulator of G-protein signalling 2, 24kD		2.06
1048_at	retinoid X receptor-gamma	1.47	1.34
36217_at	serine/threonine kinase 38	1.54	
41544_at	serum-inducible kinase		1.16
32447_at	SF-1; Steroidogenic factor-1	8.76	
36487_at	short stature homeobox 2,		-1.46
41222_at	signal transducer and activator of transcription 6 (STAT6)	1.44	
1955_s_at	SMAD6 (inhibits BMP/Smad1 (MADH1) signalling)	1.19	
37718_at	SNF-1 related kinase	1.49	1.19
35883_at	Spi-B		-2.80
1244_at	Stat2		-1.12
506_s_at	Stat5A		1.16
38994_at	STAT-induced STAT inhibitor-2		1.25
38669_at	Ste20-related serine/threonine kinase	1.24	1.65
37152_at	steroid hormone receptor superfamily		1.19
35844_at	syndecan 4		1.37
38374_at	TEIG; TGFB inducible early growth response	1.18	
38427_at	TEIG; TGFB inducible early growth response		1.38
32080_at	tetracycline transporter-like protein		1.41
224_at	TGFB inducible early growth response; TIEG	1.26	
36940_at	TGFB1-induced anti-apoptotic factor 1	1.22	1.60
32217_at	TGF-beta induced apotosis protein 12	1.40	
41445_at	TGF-beta precursor	1.14	
1890_at	TGF-beta superfamily protein	1.74	
40631_at	Tob	-1.14	1.59
39358_at	transcriptional co-repressor nuclear receptor co-repressor 2		1.42
1385_at	transforming growth factor induced protein		1.36
1830_s_at	transforming growth factor-beta		1.17
1767_s_at	transforming growth factor-beta 3 (TGF-beta 3)	-1.71	-1.63
40581_at	TRIO: triple functional domain (PTPRF interacting)	1.65	1.56

TABLE 14
Gene-Profiling Analysis of Salmon Calcitonin and PTS893 in Bone

GeneChip® Expression Probe Set Identifier	Coding Gene	Fold Increase Salmon Calcitonin	Fold Increase PTS893
32272_at	tubulin alpha	-1.20	
685_f_at	tubulin alpha isotype H2-alpha	-4.36	-1.79
330_s_at	tubulin alpha, 1,	-1.80	-1.15
151_s_at	tubulin beta	-1.40	
39399_at	tubulin beta cofactor D	-1.85	
471_f_at	tubulin beta, 4	-1.38	
40567_at	tubulin, alpha 3	-1.39	
709_at	tubulin, beta 3	-1.18	
33678_i_at	tubulin, beta, 2	-1.15	
33679_f_at	tubulin, beta, 2	-1.31	
1651_at	ubiquitin carrier protein E2-C	-3.74	-1.22
32548_at	unactive progesterone receptor		-1.33
1953_at	vascular endothelial growth factor	1.40	1.20
36101_s_at	vascular endothelial growth factor	1.45	1.44
36140_at	Y box binding protein-1	2.30	5.49

- numbers = fold down-regulated
+ numbers = fold up-regulated

[0069] *Real-time PCR.* Based on the DNA microarray data a set of transcripts was chosen for quantitative analysis by real time-PCR (RT-PCR).

[0070] Briefly, the method exploits the SyBr Green dye which intercalates into double stranded DNA. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the SyBr Green dye. Reactions are characterised by the point in time during cycling when amplification of a PCR product is first detected rather than the amount of PCR product accumulated after a fixed number of cycles. The higher the starting copy number of nucleic acid target, the sooner a significant increase in fluorescence is observed.

[0071] From each RNA sample, cDNA was made using an Applied Biosystem kit (Applied Biosystems # N808-0234) following the recommendation of the manufacturer. The PCR mixture was prepared using the SyBr Green Universal PCR Master Mix (Applied Biosystems # 4309155) as follows: 5 µl cDNA template, 400 nM of each primer, 0.2 mM deoxynucleotide triphosphates, 1 mM MgCl2 and 0.5 U Taq DNA polymerase, 5 µl SyBr Green PCR buffer and RNase free water up to a final volume of 50 µl. The PCR was performed using the ABI Prism 7700 Sequence Detection System, after a step at 95°C for 10 min, the step-cycle program was performed for a total of 40 cycles as follows: 95°C for 30 s,

60°C for 1 min. A negative control was included: PCR reaction mixture with water in place of the cDNA sample.

[0072] The initial template concentration was determined based on the threshold cycle. The threshold cycle is the PCR cycle at which fluorescence is first detected above background and has been shown to be inversely proportional to the number of target copies present in the sample. Quantification was performed by calculating the unknown target concentration relative to an absolute standard and by normalizing to a validated endogenous control such as a housekeeping gene (β-actin). Results are presented as percentage of control, once the ratio between the numbers of molecule for the gene of interest divided by the number of molecule for beta-actin has been calculated.

[0073] Based on the DNA microarray data the following set of transcripts was chosen for quantitative analysis by RT-PCR: adhesion receptor CD44, angiopoietin, bone morphogenetic protein 5, carbonic anhydrase II, cartilage oligomeric matrix protein, cathepsin K, osteopontin, pre-pro-alpha-2 type I collagen, Spi-B and Y-box binding protein.

TABLE 15
Real Time PCR Results

GeneChip® Expression Probe Set Identifier	Coding Gene	Treatment Effect Salmon Calcitonin (% respect to control)	Treatment Effect PTS893 (% respect to control)
1372_at	adhesion receptor CD44	No change	No change
1929_at	angiopoietin-1	No change	No change
1831_at	bone morphogenetic protein 5	+16	+18
40095_at	carbonic anhydrase II	- 60	No change
40161_at	cartilage oligomeric matrix protein	+34.23	No change
128_at	cathepsin K	+67.2	No change
2092_s_at	osteopontin	No change	No change
32306_g_at	pre-pro-alpha-2 type I collagen	+38	+62
35883_at	Spi-B	-44	-18
36140_at	Y-box binding protein (bone)	+14	+26
36140_at	Y-box binding protein (kidney)	+15	n.a.
36140_at	Y-box binding protein (muscle)	-26	n.a.

n.a.: not applicable

[0074] RT-PCR confirmed in most of the cases the changes observed in the gene profiling analysis, as it was the case for bone morphogenetic protein 5, carbonic anhydrase II, cathepsin K, cartilage oligomeric matrix protein, pre-pro-alpha-2 type I collagen, Spi-B and Y-Box binding protein. No changes were however detected in the level of expression of adhesion receptor CD44, angiopoietin-1 and osteopontin.

[0075] *Analysis.* Calcitonin is known to exert an effect on the differentiation, survival and resorptive activity of osteoclasts, resulting in a decreased osteoclastic activity. Pondel M, *Intl. J. Exp. Pathol.* 81(6): 405-22 (2000). These effects could be reconstructed by multi-organ gene profiling (TABLE 16).

TABLE 16
Effects on Osteoclasts

Function	Coding genes	Salmon Calcitonin	PTS893
Osteoclast determination, survival and differentiation	PU.1 (SPI1)	B, K, P, T	B
	Granulocyte to macrophage colony-stimulating factor (CSF1)	B, K	B
	Monocyte to macrophage differentiation associate (MMD)	B, K, T	B
	Osteoclast stimulating factor 1 (Autocrine stimulation of osteoclast resorptive activity)	B, K, L, P	
Bone resorption by osteoclast	H+ ATP-ases	ALL	B
	Carbonic anhydrase I, II.	B, L, P	
	Cathepsin K	ALL	
	ODF/OPGL: osteoprotegerin ligand		B
Osteoclast motility	Tubulins	ALL	
	PAK4 protein	B, M, P	

Multi-organ gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[0076] Salmon calcitonin seems to exert a paracrine regulation of the osteoclast resorptive activity, through the regulation of cystatin expression in the osteoblast, as shown in TABLE 17.

TABLE 17
Gene Expression Profiling: Osteoclast Function

GeneChip® expression probe set identifier	Coding Gene	Average controls	Average sCT	Fold change
40729_s_at	ATPase, H+ transporting, lysosomal (vacuolar proton pump) subunit G isoform 2	204	327	1.6
37367_at	ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E isoform 1	272	328	1.2
40568_at	ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunit B, isoform 2	938	1132	1.21
39241_at	carbonic anhydrase I	1266	441	-2.87
128_at	cathepsin K (pycnodysostosis)	5690	7821	1.37
129_g_at	cathepsin K (pycnodysostosis)	5036	6757	1.34
38466_at	cathepsin K (pycnodysostosis)	5494	7267	1.32
36611_at	acid phosphatase 1, soluble	254	331	1.3

[0077] PU.1 is involved in the initial stages of osteoclastogenesis. Tondravi MM *et al., Nature* 386(6620): 81-4 (1997). CSF-1 is imperative for macrophage maturation; it binds to its receptor c-*fms* on early osteoclast precursors, providing signals required for their survival and proliferation. Teitelbaum SL, *Science* 289(5484):1504-1508 (2000).

[0078] Interestingly, PTS893 also regulates the genes implicated in osteoclast differentiation and survival, SPI1, CSF-1 and MMD. This osteoclast regulation has not been previously described.

[0079] Salmon calcitonin was shown to regulate the expression of the gene coding for osteoclast stimulating factor (OSF), which is an intracellular protein produced by osteoclasts that indirectly induces osteoclast formation and bone resorption. Reddy S *et al., J. Cell Physiol.* 177 (4): 636-45 (1998). This would imply an autocrine effect of salmon calcitonin in the regulation of the osteoclast function, which is described here for the first time.

[0080] In addition, salmon calcitonin seems to exert a paracrine regulation of the osteoclast resorptive activity, through the regulation of cystatin expression in the osteoblast. Carbonic anhydrase I, II, H^+-ATPases and cathepsin K are the main effectors for dissolving bone mineral and matrix degradation. Blair HC *et al., Biochem.* (2002). Regulation of tubulins and PAK4 genes can be related to the effect of calcitonin on osteoclast motility PAK 4. Zaidi M *et al., Bone* 30(5): 655-63 (2002); Jaffer ZM & Chernoff J, *Intl. J. Biochem. Cell Biol.* 34(7): 713-7 (2002).

[0081] These results show modulating effects of calcitonin on genes affecting the direct, autocrine, paracrine and endocrine regulation of the osteoblast function (TABLE 18). These data support the hypothesis that attributes a bone anabolic effect to calcitonin.

TABLE 18
Effects on Osteoblasts

Function	Coding Gene	Salmon Calcitonin	PTS893
Antagonists of cathepsins; antiresorptive activity	Cystatins	B	
Autocrine/paracrine regulation of osteoblast function	Alpha-2-HS-Glycoprotein	B, K, T	
	Bone Morphogenetic Proteins	ALL	B
	Fibroblast Growth Factors	B, K, M, P, T	B
	IL6/LIF		B
	Insulin–like Growth Factors	ALL	B
	TGFs	B, K, M, P	B
	Tob	B, M, P	B
	Vascular Endothelial Growth Factor	B, M	X
Endocrine regulation of osteoblast function	Activin	B, L, M, P	B
	Oestrogen receptor	ALL	
	Retinoic receptor X	B, P	B
	Steroidogenic factor	B, L, P, T	
	nuclear receptors (steroid/thyroid family)		B
Transcription factor that regulates collagen type 1 synthesis	Y-box binding protein	B, K, M, P	B

Multi-organ gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[0082] The results of this EXAMPLE show modulating effects of calcitonin on genes affecting the direct, autocrine, paracrine and endocrine regulation of the osteoblast function. These data support the hypothesis that attributes a bone anabolic effect to calcitonin.

[0083] Three families of growth factors, the transforming growth factor betas (TGF-βs), insulin-like growth factors (IGFs), and bone morphogenetic proteins (BMPs), are considered to be principal local regulators of osteogenesis. Bone morphogenetic proteins are thought to

have their major effects on early precursor bone cell replication and osteoblast commitment. In contrast, TGB-βs are thought to be the most potent inducers of committed bone cell replication and osteoblast matrix production, while IGFs appear to integrate and extend the effect of both factors. McCarthy TL *et al., Crit. Rev. Oral Biol. Med.* 11(4): 409-22 (2000). These results support the fact that both salmon calcitonin and PTS893 are able to regulate these local and systemic factors implicated in bone metabolism.

[0084] The fact that salmon calcitonin regulates α2-HS glycoprotein (AHSG), which blocks TGF-β-dependent signalling in osteoblastic cells, also supports this role. Mice lacking AHSG display growth plate defects, increased bone formation with age, and enhanced cytokine-dependent osteogenesis. Szweras M *et al., J. Biol. Chem.*, 277(22): 19991-19997 (2002).

[0085] Salmon calcitonin and PTS893 were also shown to modulate the expression of the genes coding for vascular endothelial growth factor (VEGF). VEGF is known for playing a key role in normal and pathological angiogenesis. The critical role of angiogenesis for successful osteogenesis during the endochondral ossification is well documented. VEGF indirectly induces proliferation and differentiation of osteoblasts by stimulating endothelial cells to produce osteoanabolic growth factors. Wang DS *et al., Endocrinology* 138(7): 2953-62 (1997). In addition, VEGF stimulates chemotactic migration of primary human osteoblasts, suggesting a functional role in bone formation and remodelling. Mayr-Wohlfahrt U *et al., Bone* 30 (3): 472-7 (2002).

[0086] The effects of parathyroid hormone on osteoblast for mediating both bone resorption and formation have been widely described. Swarthout JT *et al., Gene* 282(1-2):1-17 (2002). It was here possible to confirm the effect of PTS893 on cytokines like interleukin 6 (IL-6), which mediates the paracrine activation of osteoclast differentiation and activity. Greenfield EM *et al., Life Sci.* 65:1087-102 (1999). PTS893 also produced a strong up-regulation on nuclear receptors (steroid/thyroid family).

TABLE 19
Gene Expression Profiling: Growth Factors and Hormones

GeneChip® expression probe set identifier	Coding Gene	Average controls	Average sCT	Fold change
39407_at	bone morphogenetic protein 1	448	607	1.36
1122_f_at	chorionic gonadotropin, beta polypeptide	263	380	1.44
39945_at	fibroblast activation protein, alpha	636	436	-1.46
1970_s_at	fibroblast growth factor receptor 2 (bacteria-expressed kinase, keratinocyte growth factor receptor, craniofacial dysostosis 1, Crouzon syndrome, Pfeiffer syndrome, Jackson-Weiss syndrome)	184	108	-1.69
32254_at	follistatin-like 3 (secreted glycoprotein)	1514	2209	1.46
38737_at	insulin-like growth factor 1 (somatomedin C)	66	37	-1.79
36782_s_at	insulin-like growth factor 2 (somatomedin A)	212	323	1.52
1591_s_at	insulin-like growth factor 2 (somatomedin A)	293	402	1.37
40422_at	insulin-like growth factor binding protein 2, 36kDa	181	105	-1.73
37319_at	insulin-like growth factor binding protein 3	495	1561	3.15
1586_at	insulin-like growth factor binding protein 3	428	722	1.69
37319_at	insulin-like growth factor binding protein 3	604	879	1.46
1586_at	insulin-like growth factor binding protein 3	355	445	1.25
1451_s_at	osteoblast specific factor 2 (fasciclin I-like) periostin	538	292	-1.84
532_at	parathyroid hormone receptor 1	1337	1849	1.38
234_s_at	pleiotrophin (osteoblast specific factor 1)	710	507	-1.4
34820_at	pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1)	422	329	-1.28
1897_at	transforming growth factor beta 1 induced transcript 1	176	296	1.68
1385_at	transforming growth factor, beta-induced, 68kDa	187	292	1.57
39588_at	tumour necrosis factor (ligand) superfamily, member 12	176	127	-1.39
31410_at	tumour necrosis factor (ligand) superfamily, member 4	197	128	-1.54
38631_at	tumour necrosis factor receptor superfamily, member 13B	134	240	1.79
35150_at	tumour necrosis factor receptor superfamily, member 5	443	298	-1.48
595_at	tumour necrosis factor, alpha-induced protein 3	118	191	1.62
1953_at	vascular endothelial growth factor	351	557	1.59
36100_at	vascular endothelial growth factor	282	407	1.45
1953_at	vascular endothelial growth factor	521	629	1.21
37268_at	vascular endothelial growth factor B	379	504	1.33
39091_at	vitamin A responsive; cytoskeleton related	421	299	-1.41

[0087] Both calcitonin and parathyroid hormone receptors belong to the G-protein receptor superfamily. After receptor stimulation, signal transduction is mediated by adenylate cyclase/cAMP/protein kinase, Phospholipase C, Phospholipase D, and MAPK (as a late effecter) pathways in the case of calcitonin, and by adenylate cyclase and phospholipase C in the case of parathyroid hormone. Gene profiling analysis allowed the reconstruction of these pathways, showing genes that were modulated by the treatment and that are localised at different levels of the signal transduction pathway.

TABLE 20
Effects on Signal Transduction and Cell Cycle

Function	Coding Gene	Salmon Calcitonin	PTS893
Signal transduction.	Adenylate cyclase		B
	Calcyclin binding protein		B
	Calreticulin	B, K, M	
	CREM	B, L, P	B
	CDC Kinase	B, M	
	MAPK	ALL	B
	Protein kinases	ALL	
	Phosphatidylinositol pathway	ALL	B
	Phosphodiesterase (IB, 4A, 4B)	ALL	B
	Phospholipase (C, D)	ALL	B
	PCNA	B	
	SMAD pathway	ALL	B
	STAT pathway	ALL	B
Cell cycle	Cyclins (A, A2, B1, C, D2, E2, G1, G2)	B	B
	Cyclin-dependent kinases 5, 6, 10	B, K, P, T	B
	Cyclin-dependent kinases inhibitor 1A, 1C, 2D)	B	B

Multi-organ gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[0088] Salmon calcitonin seems also to exert a direct influence on cell cycle, since changes in cyclins and cyclin-related proteins could be also observed, as shown in TABLE 21.

TABLE 21
Gene Expression Profiling: Signal Transduction

GeneChip® expression probe set identifier	Coding Gene	Average controls	Average sCT	Fold change
769_s_at	annexin A2	8393	6969	-1.2
32066_g_at	cAMP responsive element modulator	168	231	1.38
40777_at	catenin (cadherin-associated protein), beta 1, 88kDa	3688	4689	1.27
40697_at	cyclin A2	212	128	-1.65
40697_at	cyclin A2	272	175	-1.56
1943_at	cyclin A2	121	83	-1.45
2020_at	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	238	135	-1.76
36650_at	cyclin D2	204	312	1.53
40225_at	cyclin G associated kinase	827	1011	1.22
31700_at	G protein-coupled receptor 35	844	591	-1.43
41074_at	G protein-coupled receptor 49	242	146	-1.66
33082_at	integrin, alpha 10	171	243	1.42
33082_at	integrin, alpha 10	228	289	1.26
33411_g_at	integrin, alpha 6	65	35	-1.86
33410_at	integrin, alpha 6	201	90	-2.22
38297_at	phosphatidylinositol transfer protein, membrane-associated	753	1006	1.34
31904_at	phosphodiesterase 2A, cGMP-stimulated	555	740	1.33
38269_at	protein kinase D2	747	1067	1.43
36008_at	protein tyrosine phosphatase type IVA, member 3	518	376	-1.38
35361_at	PTEN induced putative kinase 1	95	255	2.69
35178_at	WNT inhibitory factor 1	1746	2127	1.22

[0089] Bone morphogenetic protein (BMP) controls osteoblast proliferation and differentiation through Smad proteins. Tob, a member of the emerging family of antiproliferative proteins, is a negative regulator of BMP/Smad signalling in osteoblasts. Smad pathway as well as Tob as one of their regulators were also identified as genes modulated by the sCT and PTS893 treatment, in agreement with the hypothesised effect of

both compounds on BMP regulation of bone remodelling. Within this context, both compounds seem to exert a direct influence on cell cycle, since changes in cyclins and cyclin-related proteins could be also observed.

[0090] Both compounds regulate also synthesis and degradation of extracellular matrix components (TABLE 22).

TABLE 22
Effects on Extracellular Matrix

Function	Coding Gene	Salmon Calcitonin	PTS893
Cell attachment. Signal transduction.	Integrins	B, M, P	B
Collagen digestion	Collagenase	B	
	Matrix metalloproteinases I, II	B, L, P, T	
Collagen synthesis	Procollagen endopeptidase/proteinase		B
	Lysyl hydroxylase		B
Extracellular matrix component	Aggrecan		B
	Cartilage Oligomeric Matrix Protein Precursor	B, K,	
	Collagen type I, type II, type III, type IV, type V, type VI, type IX, type X, type XI, type XIII, type XIV, type XV, and/or type XVI)	ALL	B
	Chondroitin sulphate proteoglycan	K, M, T	B
	Dermatopontin		B
	Heparan sulphate proteoglycan	L,T	B
	Syndecan		B

Multi-organ gene expression profiling in salmon calcitonin-treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[0091] Salmon calcitonin regulates also the synthesis and degradation of extracellular matrix components, as shown in TABLE 23.

TABLE 23
Gene Expression Profiling: Extracellular Matrix

GeneChip® expression probe set identifier	Coding Gene	Average controls	Average sCT	Fold change
36253_at	bone gamma-carboxyglutamate (gla) protein (osteocalcin)	26305	33265	1.26
32094_at	carbohydrate (chondroitin 6) sulfotransferase 3	253	130	-1.95
32094_at	carbohydrate (chondroitin 6) sulfotransferase 3	292	241	-1.21
41447_at	carbohydrate (chondroitin) synthase 1	192	107	-1.79
34042_at	chondroadherin	7965	10266	1.29
32306_g_at	collagen, type I, alpha 2	7740	9337	1.21
32488_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	2399	1294	-1.85
34802_at	collagen, type VI, alpha 2	2374	1500	-1.58
35816_at	cystatin B (stefin B)	983	1897	1.93
34029_at	dentin matrix acidic phosphoprotein	216	587	2.72
38059_g_at	dermatopontin	695	962	1.38
38057_at	dermatopontin	1090	1381	1.27
33929_at	glypican 1	235	163	-1.44
39350_at	glypican 3	64	50	-1.29
37176_at	hyaluronoglucosaminidase 1	109	266	2.43
1546_at	hyaluronoglucosaminidase 1	49	79	1.59
36683_at	matrix Gla protein	65	117	1.8
609_f_at	metallothionein 1B (functional)	2693	3485	1.29
870_f_at	metallothionein 3 (growth inhibitory factor (neurotrophic))	1744	2296	1.32
38307_at	neurochondrin	452	696	1.54
34342_s_at	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I)	16370	21279	1.3
38127_at	syndecan 1	534	346	-1.54
1693_s_at	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	4549	5522	1.21
2092_s_at	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	7748	9576	1.24
38308_g_at	neurochondrin	679	490	-1.39

[0092] Of particular interest is the regulation of the Y-Box binding protein (YB-1), which appears to be modulated by both treatments and in four out of six organs analysed in the salmon calcitonin group. YB-1 is a protein that interacts with a TGF-β response element in the distal region of the collagen alpha 1(I) gene. YB-1 protein activates the collagen promoter and

translocates into the nucleus during TGF-β addition to fibroblasts, suggesting a role for this protein in TGF- β signalling. Sun W *et al., Matrix Biol.* 20(8): 527-41 (2001).

[0093] In addition, salmon calcitonin and PTS893 regulated some aspects of the mineralization of the bone extracellular matrix, since changes in amelogenin, dentin and ectonucleotide pyrophosphatases were observed.

TABLE 20
Effects on Mineralization and Visualisation

Function	Coding Gene	Salmon Calcitonin	PTS893
Cement component	Amelogenin	B, L	B
Mineral matrix protein	Dentin	B	B
Enzyme for synthesis of inorganic Pi	Ectonucleotide pyrophosphatases	B, M	
Growth factor vascularisation	VEGF	B, M	B

Multi-organ gene expression profiling in salmon calcitonin treated animals. Organs where changes in expression were seen are displayed. B= bone; K= kidney; M= muscle; P= pituitary; L= liver; T= trachea.

[0094] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes. In addition, all GenBank accession numbers, Unigene Cluster numbers and protein accession numbers cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each such number was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[0095] The present invention is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the invention. Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatus within the scope of the invention, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications and variations are intended to fall within the scope of the appended claims. The present invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.